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Shahan Islam

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

2550/KIP

Applicant:	Norio MIURA, Noboru YAMAZOE, Taizo UDA	
Serial	No.:08/985,007	Examiner: Christopher L. Chin
Filed:	December 4, 1997	Group Art Unit: 1641
For:	Apparatus for Measuring a Medical Substance; a Sensor for Use in the Apparatus and a Sensing Element for Use in the Sensor	

BRIEF ON APPEAL

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I. STATUS OF CLAIMS

Claims 14 to 27 remain.

The application was filed on December 4, 1997 with claims 1 to 13.

Claims 1 to 12 were rejected in an Office Action dated January 22, 1999 and claim 13 was withdrawn from consideration.

Claims 1 to 12 were cancelled and claims 14 to 27 were added in an Amendment dated May 24, 1999.

Claims 14 to 27 were rejected in Final Action dated August 17, 1999.

Claims 14 and 19-24 were amended in an Amendment after Final Action filed on December 10, 1999.

However, the proposed amendment was deemed to raise new issues according to an Advisory Action dated December 27, 1999.

Accordingly, the status of the claims is as they were after the filing of the May 24, 1999 amendment.

II. STATUS OF AMENDMENTS

Original claims 1 to 12 were rejected in an Office Action dated January 22, 1999 and claim 13 was withdrawn from consideration.

In an Amendment dated May 24, 1999, claims 1 to 12 were cancelled and claims 14 to 27 were added.

Claims 14 to 27 were rejected in "Final Action" dated August 17, 1999.

In an Amendment dated December 10, 1999 after the Final Rejection, claims 14 and 19-24 were amended. However, entry of the amendment was denied as raising new issues as stated in the Advisory Action dated December 27, 1999.

Accordingly, the status of the claims is as they were after the filing of the May 24, 1999 amendment.

III. SUMMARY OF THE INVENTION

The present invention relates to a medical substance measuring apparatus in which a medical substance is measured by using a resonance phenomenon resonating with an evanescent wave and related to a medical substance sensor for use in the apparatus. A major feature of the present invention is that the medical substance to be measured by the apparatus is fixed to a resonance material as an antigen.

In order to measure the medical substance (an antigen) contained in a sample using a resonance phenomenon resonating with an evanescent wave, the apparatus comprises:

a resonance phenomenon generating section having a resonance material; and

a detecting means for detecting a change of an incident light which is made incident upon said resonance material to generate said resonance phenomenon or a change of a reflected light thereof; and

wherein the medical substance (antigen) to be measured is fixed to said resonance material.

Further, the present invention is also directed to a method for measuring the medical substance (an antigen) contained in a sample using a resonance phenomenon resonating with an evanescent wave. The method comprises the steps of:

fixing a medical substance (an antigen) to be measured to a resonance material wherein the resonance phenomenon is caused to resonate with an evanescent wave;

mixing an antibody with said sample wherein the antibody is coupled with said medical substance (antigen) in a specific manner;

bringing a mixture of said antibody and said sample in contact with the resonance material to which said medical substance (antigen) has been fixed;

making a light incident upon said resonance material;

detecting a change of the incident light or a change of a reflected light thereof when said resonance phenomenon is generated; and

recognizing an amount of said medical substance (antigen) contained in said sample on the basis of said change of the incident light or the reflected light.

IV. STATEMENT OF ISSUES ON APPEAL

(1) Whether claims 14 to 27 are patentable under 35 U. S. C. § 112, second paragraph.

(2) Whether claims 14, 15, 17, 19, 22, 24, 25 and 27 are patentable under 35 U. S. C. § 102 over Batchelder (U.S. Patent No. 4,844,613), Finlan (U.S. Patent Nos. 4,997,278 and 5,047,213), and Stewart (U.S. Patent No. 5,229,833).

(3) Whether the drawings are allowable as they show every feature of the claimed invention under 37 CFR 1.83 (a).

V. GROUPING OF CLAIMS

Although independent claims 14, 22 and 24 show the main features of the invention, dependent claims 15 to 21, 23, and 25 to 27 are believed to be separately patentable.

VI. ARGUMENT

This is a brief supporting an appeal from Final Rejection dated August 17, 1999, in which:

(a) claims 14 to 27 were rejected under 35 U.S.C. § 112, second paragraph;

(b) claims 14, 15, 17, 19, 22, 24, 25 and 27 were rejected under 35 U.S.C. § 102 over Batchelder, Finlan and Stewart; and

(c) the drawings were objected to because they do not show every feature of the claimed invention under 37 CFR 1.83 (a).

In the Advisory Action, the Examiner deemed that the amendment dated December 10, 1999 after Final Action raised new issues and therefore did not enter them. Accordingly, the status of the claims is as they were upon the submission of the first amendment dated May 24, 1999.

Applicant respectfully submits, however, that the new issue ruling was in error.

This brief is accordingly divided as follows:

(a) Part I demonstrates that the rejection in view of the May 24 Amendment was in error and that claims 14- 27 therein violate neither Sections 112 nor 102; and

(b) alternatively, Part II demonstrates that:

(i) claims 14-27 as presented in the Amendment After Final satisfy the requirements of Sections 112 and 102; and

(ii) the new issue determination in the Advisory Action was erroneous.

PART I - THE REJECTION IN VIEW OF THE MAY 24 AMENDMENT WAS IN ERROR AS CLAIMS 14- 27 THEREIN VIOLATE NEITHER SECTIONS 112 NOR 102

A. THE §112 REJECTION SHOULD BE WITHDRAWN

Claims 14-27 were rejected as being unpatentable under 35 U. S. C. § 112, second paragraph. Such rejection should be withdrawn for the following reasons.

First, the Examiner pointed out that it was not clear from the last line of claim 14 whether the antigen fixed to the resonance material is "a medical substance," or a reagent for detection of the medical substance. The last line of claim 14 in the May 24 Amendment reads as follows:

> "wherein **the medical substance** to be measured is fixed to said resonance material **as an antigen**."

Therefore, claim 14 clearly defines that the antigen fixed to the resonance material is "a medical substance," not a reagent for detection of the medical substance.

Further, the Examiner asserts that, "if the antigen is the 'medical substance', then a corresponding antibody specific for the antigen would have to be present on the resonance material to permit detection of the antigen." It is respectfully submitted that the Examiner's assertion is proper for the conventional detecting technology. However, as described above, the present invention has, as a feature, that not the antibody but the antigen i.e., medical substance is fixed to the resonance material. This is vastly different from conventional technology, because such construction makes it easy to detect the medical substance having a very low molecular weight. The antibody specific for the antigen is therefore not required on the resonance material.

The Examiner rejected claim 22 as it suffers from the same deficiency as claim 14. Applicant respectfully submits however that the rejection of claim 22 should be withdrawn for the same reasons as mentioned above for claim 14.

Next, the Examiner rejected claims 19-21 because claim 19 depends on canceled claim 2. Applicant has corrected the errors in the December 10 Amendment to depend claim 19 properly on claim 14.

Furthermore, the Examiner asserts that, in claims 20 and 23, it is not clear as to how the antigen can be fixed to a surface of the metal film which is opposite to the surface prism when

the metal film is formed on the surface of the prism, and therefore that the claims fail to recite the presence of another metal film that is positioned opposite the metal film on the surface of the prism to support the antigen. However, with respect to the structures of the medical substance, metal film and prism, claims 20 and 23 recite that "**the medical substance** to be measured is **fixed** as an antigen **to another surface of said metal film which is opposite to the surface on which said prism is formed**."

Accordingly, such an Examiner's understanding is incorrect, because there is no metal film that is positioned opposite the metal film on the surface of the prism to support the antigen and it is therefore not necessary to recite the presence of another metal film.

Therefore, though the Examiner objected to the drawings for the reason that the antigen fixed to another metal film in claims 20 and 23 must be shown, the objection to the drawings should be withdrawn, for the reasons stated above.

Further, the Examiner asserts that claim 24 is vague as to whether the antigen is "a medical substance," or a reagent for detection of the medical substance. Lines 4-5 of claim 24 in the May 24 Amendment read as follows:

> "fixing **a medical substance** to be measured to a resonance material wherein a resonance phenomenon is caused to resonate with an evanescent wave **as an antigen.**"

Therefore, claim 24 clearly defines that the antigen is "a medical substance," not a reagent for detection of the medical substance, as explained above for claim 14.

Also, the Examiner asserts that lines 6-7 of claim 24 are not clear as to whether the antibody is coupled with the medical substance or the sample, and is specific for the antigen or the medical substance. Lines 6-7 of claim 24 in the May 24th Amendment read as follows:

"mixing **an antibody which is coupled with said fixed medical substance in a specific manner** to said sample."

Therefore, claim 24 clearly defines that the antibody is coupled with the medical substance and is specific for the medical substance which is the antigen.

With respect to the mixture recited in lines 8-9 of claim 24, the Examiner deems it to lack antecedent support and to be redundant because the sample and antibody are already in contact with the resonance material. Lines 8-9 of claim 24 in the May 24 Amendment read as follows:

"bringing the mixture in contact with the resonance material to which said medical substance has been fixed."

The mixture recited in lines 8-9 of claim 24 means the mixture of said antibody and said sample defined in lines 6-7. Further, when mixing the antibody and the sample, some of the antibody is coupled with said medical substance contained in the sample in a specific manner, and when the mixture is brought in contact with the resonance material to which the medical substance has been fixed, the rest of the antibody is coupled with the fixed medical substance in a specific manner. Therefore, the step recited in lines 8-9 of claim 24 is not redundant but an essential step of the claimed invention.

Further, the Examiner asserts that lines 11-12 of claim 24 are vague because any change in the properties of the incident light is the result of "medical substance" and antibodies being bound to the resonance material which is not clearly set forth in the detection step recited in these two lines. However, lines 11-12 of claim 24 read as follows:

"detecting a change of the incident light or a change of a reflected light thereof when said resonance phenomenon is generated;"

Claim 24 therefore clearly defines that a change of the incident light or a change of a reflected light thereof is detected by using the resonance phenomenon generated by the previous steps in lines 1-10 when an antibody-antigen reaction is caused.

In view thereof, all of the above-mentioned claims particularly point out and distinctly claim the subject matter of the invention. Such rejection under §112 should therefore be withdrawn and all of the above-mentioned claims are allowable.

Each remaining claim is dependent directly or indirectly on claims 14, 22 and 24, and is also allowable for the same reasons.

B. THE REJECTION IN VIEW OF THE REFERENCES SHOULD BE WITHDRAWN

The Examiner asserted claims 14, 15, 17, 19, 22, 24, 25 and 27 are unpatentable under 35 U. S. C. § 102 because the claims are anticipated by Batchelder (U.S. Patent No. 4,844,613), Finlan (U.S. Patent Nos. 4,997,278 and 5,047,213), and Stewart (U.S. Patent 5,229,833). As demonstrated herein, the rejection of the May 24 Amendment was in error and the rejected claims do not violate Section 102.

1. BATCHELDER DOES NOT DISCLOSE, TEACH OR OTHERWISE SUGGEST MANY EXPLICITLY RECITED CLAIM FEATURES

Batchelder et al (U.S. Patent No. 4,844,613) is directed to an optical sensor device for detecting the presence of a specific material by using surface plasmon resonance phenomenon. A transparent body is coated with a thin gold film which film may be coated with an antibody (see abstract).

In contrast to Batchelder et al. in the claimed invention, it is not an antibody but **antigen** fixed to a surface of a metal film. The reference does not disclose, teach or otherwise suggest a measuring apparatus or sensor where an antigen to be measured is fixed to a surface of a metal film or a resonance material as recited in independent claims 14, 22 and 24.

Nonetheless, the Examiner asserts that the current claims do not clearly define the differences between the claimed invention and the reference, *i.e.*, the presence of an antigen on the resonance material, wherein the antigen is the analyte to be detected.

However, the last two lines of claims 14 and 22 in the May 24 Amendment read as follows:

> "wherein **the medical substance to be measured is fixed to said resonance material as an antigen.**"

Therefore, claims 14 and 22 clearly define that the medical substance fixed to the resonance material is "an antigen."

Further, lines 4-5 of claim 24 in the May 24 Amendment read as follows:

> **"fixing a medical substance to be measured to a resonance material** wherein a resonance phenomenon is caused to resonate with an evanescent wave **as an antigen.**"

Therefore, claim 24 clearly defines that the medical substance fixed to the resonance material is the antigen. Thus, the claimed invention clearly defines the difference from the reference, *i.e.*, the presence of an antigen on the resonance material.

Further, according to the present invention, such a medical substance having an extremely small molecular weight, which is difficult to detect by the conventional technique, can be easily detected and is thus clearly non-obvious over the prior art.

As mentioned in the specification on page 4, line 28 to page 5, line 24, according to previously known methods, it has been tried to measure a medical substance wherein an antibody is fixed to a resonance material, such as a metal thin film, and the medical substance which is coupled to the antibody in a specific manner is detected directly. However, the medical substance contained in a body liquid, such as urine or blood, has a significantly small molecular

weight; therefore, even if the medical substance reacts with the antibody fixed to the resonance material, the change of the resonance angle, etc. is extremely small. Therefore, it is very difficult to detect such a medical substance by conventional methods.

By contrast, the present invention provides an apparatus for detecting a medical substance using a resonance phenomenon resonating with an evanescent wave, by which even a medical substance having a small molecular weight can be detected. In accordance with the present invention, a medical substance to be measured by the apparatus is previously fixed to the resonance material as an antigen; a known amount of an antibody is mixed in a sample; the antibody is brought into contact with the resonance material on which the antigen (medical substance to be detected) has been fixed; then the change of the condition for generating the resonance phenomenon (resonance angle, etc.) is observed when an antigen-antibody reaction is caused. Since an antibody has a significantly greater molecular weight, when such an antibody is coupled with the antigen (medical substance to be detected) fixed on the resonance material, the change of the condition, i.e. resonance angle, etc. is sufficiently great to be detected. It should be noted that the amount of the antibody mixed in the sample is previously known, so that the amount of the medical substance contained in the sample can be indirectly calculated from the change of the condition. Therefore, according to the invention, a sufficient sensitivity can be obtained in the measurement of a medical substance having an extremely small molecular weight contained in a sample, because the antibody, which is coupled with the antigen (medical substance) fixed to the resonance material, has a sufficient molecular weight to largely change the resonating condition. This is clearly proved in the embodiments mentioned in the original specification.

Therefore, inasmuch as the present invention has a distinctive feature that not an antibody but an antigen is fixed on the resonance material, this is totally different from the conventional technology, because such construction makes it easy to detect the medical substance having a very small molecular weight.

In view thereof, Batchelder clearly teaches away from the invention. See, e.g., In re Hedges, 783 F.2d 1038 (Fed. Cir. 1986) (Teaching away from the invention by the prior art is indicative of non-anticipation and non-obviousness). Monarch Knitting Machinery Corp. v. Fukuhara Industrial & Trading Co. Ltd., 139 F.3d 977 (Fed. Cir. 1998).

Therefore, independent claims 14, 22 and 24, which recite the distinctive features, are not anticipated by Batchelder and thus they are allowable.

Each remaining claim is dependent, directly or indirectly, on claims 14, 22 and 24, and is also allowable for the same reasons.

2. **FINLAN AND STEWART FAIL TO TEACH OR SUGGEST THE INVENTION**

The Examiner further asserted claims 14, 15, 17, 19, 22, 24, 25 and 27 are unpatentable under 35 U. S. C. § 102 because the claims are anticipated by Finlan et al (U.S. Patent Nos. 4,997,278 and 5,047,213), and Stewart (U.S. Patent No. 5,229,833).

However, Finlan et al (U.S. Patent Nos. 4,997,278 and 5,047,213) are directed to sensors using the principle of surface plasmon resonance (SPR) to monitor the progress of the reaction between a sample and a sensitive layer, for example an antibody layer (see abstracts).

Accordingly, Finlan does not disclose, teach or otherwise suggest the distinctive feature of the claimed invention that the antigen is fixed on the resonance material.

Similarly, Stewart also fails to teach the above characteristics of the invention. Stewart is related to an optical sensor. A resonance mirror device used in the optical sensor consists of a prism structure onto which one low and one high index dielectric film is deposited. In

accordance with Stewart, antibodies for the species to be detected are immobilized onto the surface of the prism, and the species bind to the antibody layer (see column 4, lines 34-56). Therefore, Stewart fails to disclose or teach the distinctive feature of the claimed invention that the antigen is fixed on the resonance material.

Lack of teaching or suggestion in the art for key claimed features is indication of non-anticipation and non-obviousness (See e.g. ACS Hospital Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572 (Fed. Cir. 1984); In re Rouffet, 149 F.3d 1350 (Fed. Cir. 1998).

In view of the above, the prior art, alone or in combination, fails to disclose, teach or even remotely suggest the invention. Accordingly, the rejection in view of the references should be withdrawn and independent claims 14, 22 and 24 are allowable.

Each remaining claim is dependent on claims 14, 22 and 24, directly or indirectly and is also allowable for the same reasons.

PART II - THE NEW ISSUE DETERMINATION WAS IN ERROR AND CLAIMS 14- 27 (AS ALTERNATIVELY PRESENTED) VIOLATE NEITHER SECTIONS 112 NOR 102

A. THE §112 REJECTION SHOULD BE WITHDRAWN

Part I, Section A. addresses, in detail, why the claims clearly define and particularly point out the subject matter of the invention. Such arguments are also applicable here, because the distinctions pointed to in claims 14-27 of the May 24 Amendment are also present in claims 14-27 of the December 10 Amendment as alternatively presented herein. Accordingly, the arguments of Part I, Section A. are incorporated herein by reference as though fully set forth.

As explained, claims 14-27 are being presented here in the alternative, because it is submitted (and demonstrated in Section II. C. infra) that the Amendment presented after the Final Action (with some minor changes to place it in better form for appeal) does not raise "new issues." The amendments are quite minor and merely clarify the invention. The sole substantive

amendment of "a medical substance" into "a medical substance (an antigen)" is only to make clear that the medical substance fixed to the resonance material is the antigen and that an antibody is coupled with the antigen in a specific manner, because the Examiner is confused with those features.

Applicant now therefore demonstrates herein how, in addition to the arguments in Part I, Section A, claims 14-27 as alternatively presented in the Amendment after the Final Action, clearly define and particularly point out the subject matter of the invention.

Claims 14-27 were rejected as being unpatentable under 35 U. S. C. § 112, second paragraph. Such rejection should be withdrawn for the following reasons.

First, the Examiner pointed out that that the last line of claim 14 is not clear as to whether the antigen fixed to the resonance material is "a medical substance," or a reagent for detection of the medical substance. The last line of claim 14 in the December 10 Amendment reads as follows:

> "wherein **the medical substance (antigen)** to be measured is fixed to said resonance material."

Claim 14 therefore clearly defines that the antigen fixed to the resonance material is "a medical substance," not a reagent for detection of the medical substance.

As the Examiner appears to assert that the current claims do not clearly define the presence of an antigen on the resonance material wherein the antigen is the analyte that is to be detected, applicant has amended claim 14 to clarify the points mentioned by the Examiner.

Further, the Examiner asserts that, "if the antigen is the 'medical substance', then a corresponding antibody specific for the antigen would have to be present on the resonance material to permit detection of the antigen." It is respectfully submitted that the Examiner's

assertion is proper for the conventional detecting technology. However, as described above, the present invention has a feature that it is not the antibody but the antigen i.e., medical substance which is fixed to the resonance material. This is vastly different from the conventional technology because such construction makes it easy to detect medical substance having a very small molecular weight. Therefore, the antibody specific for the antigen is not required on the resonance material.

The Examiner rejected claim 22 as it suffers from the same deficiency as claim 14. However, Applicant respectfully submits that the rejection of claim 22 should be withdrawn for the same reasoning as mentioned above for claim 14.

Next, the Examiner rejected claims 19-21 because claim 19 depends on canceled claim 2. Accordingly, Applicant corrected the errors in the December 10 Amendment to properly depend claim 19 on claim 14.

Furthermore, the Examiner asserts that, in claims 20 and 23, it is not clear how the antigen can be fixed to a surface of the metal film which is opposite to the surface prism when the metal film is formed on the surface of the prism and therefore that the claims fail to recite the presence of another metal film that is positioned opposite the metal film on the surface of the prism to support the antigen. However, with respect to the structures of the medical substance, metal film and prism, claims 20 and 23 recite that "**the medical substance (antigen)** to be measured is **fixed to a surface of said metal film**."

Accordingly, it is submitted that the Examiner's understanding is incorrect, because there is no metal film that is positioned opposite the metal film on the surface of the prism to support the antigen and therefore it is not necessary to recite the presence of another metal film.

Therefore, though the Examiner objected to the drawings for the reason that the antigen fixed to another metal film in claims 20 and 23 must be shown, the objection to the drawings should be withdrawn, for the same reasons stated above.

Further, the Examiner asserts that claim 24 is vague as to whether the antigen is "a medical substance," or a reagent for detection of the medical substance. Lines 4-5 of claim 24 in December 10 Amendment read as follows:

> "fixing **a medical substance (antigen)** to be measured to a resonance material wherein the resonance phenomenon is caused to resonate with an evanescent wave."

Therefore, claim 24 clearly defines that the antigen is "a medical substance," not a reagent for detection of the medical substance, as explained above for claim 14.

Also, the Examiner asserts that lines 6-7 of claim 24 are not clear as to whether the antibody is coupled with the medical substance or the sample, and is specific for the antigen or the medical substance. Lines 6-7 of claim 24 in the December 10 Amendment read as follows:

> **"mixing an antibody with said sample wherein the antibody is coupled with said medical substance (antigen) in a specific manner."**

Therefore, claim 24 clearly defines that the antibody is coupled with the medical substance (antigen) and also is specific for the medical substance (antigen).

With respect to the mixture recited in lines 8-9 of claim 24, the Examiner deems it to lack antecedent support and to be redundant because the sample and antibody are already in contact with the resonance material. Lines 8-9 of claim 24 in December 10 Amendment read as follows:

> **"bringing a mixture of said antibody and said sample in contact with the resonance material to which said medical substance (antigen) has been fixed."**

The mixture recited in lines 8-9 of claim 24 means the mixture of said antibody and said sample defined in lines 6-7. Further, when mixing the antibody and the sample, some of the antibody is coupled with said medical substance contained in the sample in a specific manner, and when the mixture is brought in contact with the resonance material to which said medical substance has been fixed, the rest of the antibody is coupled with the fixed medical substance in a specific manner. Therefore, the step recited in lines 8-9 of claim 24 is not redundant but an essential step of the claimed invention.

Further, the Examiner asserts that lines 11-12 of claim 24 are vague because any change in the properties of the incident light is the result of "medical substance" and antibodies being bound to the resonance material which is not clearly set forth in the detection step recited in these two lines. However, lines of 11-12 of claim 24 read as follows:

> **"detecting a change of the incident light or a change of a reflected light thereof when said resonance phenomenon is generated."**

Therefore, claim 24 clearly defines that a change of the incident light or a change of a reflected light thereof is detected by using the resonance phenomenon generated by the previous steps in lines 1-10 when an antibody-antigen reaction is caused.

In view thereof, all of the above-mentioned claims particularly point out and distinctly claim the subject matter of the invention. Therefore, such rejection under §112 should be withdrawn and all of the above-mentioned claims are allowable.

Each remaining claim is dependent directly or indirectly on claims 14, 22 and 24, and is also allowable for the same reasons.

B. THE REJECTION IN VIEW OF THE REFERENCES SHOULD BE WITHDRAWN

The Examiner asserted claims 14, 15, 17, 19, 22, 24, 25 and 27 are unpatentable under 35 U. S. C. § 102 because the claims are anticipated by Batchelder (U.S. Patent No. 4,844,613), Finlan (U.S. Patent Nos. 4,997,278 and 5,047,213), and Stewart (U.S. Patent No. 5,229,833).

In Part I, Section B., applicant fully explained the differences between the claimed invention and the prior art. Such arguments are also applicable here, because the distinctive features pointed to in claims 14-27 of the May 24 Amendment are also present in claims 14-27 of the December 10 Amendment as alternatively presented herein. The Amendment presented after the Final Action does not raise any new issues as it has only some minor changes to place it in better form and to clarify the Examiner's points. The sole substantive amendment of "a medical substance----as an antigen" into "a medical substance (antigen)" is only to make clear that the medical substance fixed to the resonance material is the antigen and that an antibody is coupled with the antigen in a specific manner, because the Examiner is confused with those features.

Accordingly, the arguments of Part I, Section B. are incorporated herein by reference as though fully set forth.

1. BATCHELDER TEACHES AWAY FROM THE INVENTION

As explained in the above Part I, Section B., Batchelder et al teaches an optical sensor device for detecting the presence of a specific material by using surface plasmon resonance phenomenon. A transparent body is coated with a thin gold film which film may be coated with an antibody (see abstract).

In contrast to Batchelder et al, in the claimed invention, an antigen is fixed to a surface of a metal film. Thus, the reference does not disclose, teach or otherwise suggest a measuring

apparatus or sensor where an antigen (a medical substance) to be measured is fixed to a surface of a metal film or a resonance material as recited in independent claims 14, 22 and 24.

Nonetheless, the Examiner asserts that the current claims do not clearly define the differences between the claimed invention and the reference, *i.e.*, the presence of an antigen on the resonance material, wherein the antigen is the analyte that is to be detected.

Accordingly, in order to make clear that the medical substance is an antigen, applicant amended the last two lines of claims 14 and 22 in the December 10 Amendment as follows:

> "wherein **the medical substance (antigen) to be measured is fixed to said resonance material.**"

Therefore, claims 14 and 22 clearly define that "the medical substance" fixed to the resonance material is "an antigen."

Further, lines 4-5 of claim 24 in the December 10 Amendment read as follows:

> "**fixing a medical substance (an antigen) to be measured to a resonance material** wherein the resonance phenomenon is caused to resonate with an evanescent wave."

Therefore, claim 24 clearly defines that the medical substance fixed to the resonance material is the antigen. Thus, the claimed invention clearly defines the differences from the reference, *i.e.*, the presence of an antigen on the resonance material.

In as much as the present invention has a distinctive feature that not an antibody but an antigen is fixed on the resonance material, this is totally different from the conventional technology, because such construction makes it easy to detect the medical substance having a very small molecular weight.

In view thereof, Batchelder clearly teaches away from the invention. Therefore, independent claims 14, 22 and 24, which recite the distinctive features, are allowable.

Each remaining claim is dependent, directly or indirectly, on claims 14, 22 and 24, and is also allowable for the same reasons.

2. FINLAN AND STEWART FAIL TO TEACH OR SUGGEST THE INVENTION

The Examiner further asserted claims 14, 15, 17, 19, 22, 24, 25 and 27 are unpatentable under 35 U. S. C. § 102 because the claims are anticipated by Finlan et al (U.S. Patent Nos. 4,997,278 and 5,047,213), and Stewart (U.S. Patent No. 5,229,833).

However, Finlan et al (U.S. Patent Nos. 4,997,278 and 5,047,213) are directed to sensors using the principle of surface plasmon resonance (SPR) to monitor the progress of the reaction between a sample and a sensitive layer, for example an antibody layer (see abstracts).

Accordingly, Finlan does not disclose, teach or otherwise suggest the distinctive feature of the claimed invention that the antigen is fixed on the resonance material.

Similarly, Stewart also fails to teach the above characteristics of the invention. Stewart is related to an optical sensor. A resonance mirror device used in the optical sensor consists of a prism structure onto which one low and one high index dielectric film is deposited. In accordance with Stewart, antibodies for the species to be detected are immobilized onto the surface of the prism, and the species bind to the antibody layer (see column 4, lines 34-56). Therefore, Stewart fails to disclose or teach the distinctive feature of the claimed invention that the antigen is fixed on the resonance material.

In view of the above, the prior art, alone or in combination, fails to disclose, teach or even remotely suggest the invention. Accordingly, the rejection in view of the references should be withdrawn and independent claims 14, 22 and 24 are allowable.

Each remaining claim is dependent on claims 14, 22 and 24, directly or indirectly and is also allowable for the same reasons.

C. THE AMENDMENTS AFTER FINAL ACTION DID NOT RAISE NEW ISSUES

In the Advisory Action, the Examiner pointed out that "the amendments to claim 14 (a positive recitation of a medical substance on the resonance material) raises new issues-112 2nd paragraph because the claimed apparatus cannot detect a medical substance in a sample when the claim now recites the medical substance as already being part of the claimed apparatus."

However, the Amendment presented after the Final Action is only to clarify the Examiner's points. The Examiner pointed out that the last line of claim 14 is not clear as to whether the antigen fixed to the resonance material is a medical substance, or a reagent for detection of the medical substance. The last line of claim 14 in the May 24 Amendment reads as follows:

> "wherein **the medical substance to be measured is fixed to said resonance material as an antigen."**

Therefore, claim 14 clearly defines that the antigen fixed to the resonance material is a medical substance, not a reagent for detection of the medical substance.

Nonetheless, the Examiner maintained the rejection in a Final Action and therefore, applicant amended claim 14 as follows:

> "wherein **the medical substance (antigen) to be measured is fixed to said resonance material."**

Thus, the sole substantive amendment of "a medical substance------as an antigen" into "a medical substance (antigen)" is only to make clear that the medical substance fixed to the resonance material is the antigen, because the Examiner is confused with this feature. Accordingly, the Amendment after the Final Action changing "as an antigen" to "(antigen)" does

not raise any new issues and should be deemed to place the application in better form for appeal by simplifying the issues for appeal.

CONCLUSION

In view of the foregoing, it is respectfully submitted that the Examiner's rejection of claims 14 to 27 be reversed.

Respectfully submitted,
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Shahan Islam, Esq. (Reg No. 32,507)

Dated: May 11, 2000

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APPENDIX I - CLAIMS

14. (as of May 24 Amendment) An apparatus for measuring a medical substance contained in a sample using a resonance phenomenon resonating with an evanescent wave, said apparatus, comprising:

a resonance phenomenon generating section having a resonance material; and

a detecting means for detecting a change of an incident light which is made incident upon said resonance material to generate said resonance phenomenon or a change of a reflected light thereof; and

wherein the medical substance to be measured is fixed to said resonance material as an antigen.

15. An apparatus according to claim 14, wherein said change to be detected by said detecting means is an incident angle of said light being made incident upon said resonance material when an intensity of the reflected light thereof is decreased.

16. An apparatus according to claim 14, wherein said change to be detected by said detecting means is a wavelength or a wave number of said reflected light when an intensity of said reflected light is decreased.

17. An apparatus according to claim 14, wherein said change to be detected by said detecting means is an intensity of said reflected light when the incident light is made incident upon said resonance material with a predetermined incident angle.

18. An apparatus according to claim 14, wherein said change to be detected by said detecting means is an incident angle of said incident light when a phase of said reflected light is varied.

19. An apparatus according to claim 2, wherein said resonance phenomenon is a surface plasmon resonance phenomenon.

20. An apparatus according to claim 19, wherein said resonance phenomenon generating section comprises a prism having a high refractive index, a thin metal film directly or indirectly formed on one of said prism as said resonance material, and a light source for making a light incident upon said metal film via said prism, wherein the medical substance to be measured is fixed as an antigen to another surface of said metal film which is opposite to the surface on which said prism is formed.

21. An apparatus according to claim 20 further comprising a calculating means for recognizing an amount of said medical substance contained in said sample in accordance with the change detected by said detecting means.

22. A medical substance sensor for use in an apparatus for measuring a medical substance contained in a sample using a resonance phenomenon resonating with an evanescent wave comprising a resonance material where a resonance phenomenon is caused to resonate with an evanescent wave, wherein the medical substance to be measured is fixed to said resonance material as an antigen.

23. A medical substance sensor according to claim 22 further comprising a prism having a high refractive index, a thin metal film which is directly or indirectly formed on one of the surfaces of said prism as said resonance material, wherein the medical substance to be measured is fixed as an antigen to another surface of said metal film which is opposite to the surface on which said prism is formed.

24. A method for measuring a medical substance contained in a sample using a resonance phenomenon resonating with an evanescent wave, said method comprising the steps of:

fixing a medical substance to be measured to a resonance material wherein a resonance phenomenon is caused to resonate with an evanescent wave as an antigen;

mixing an antibody which is coupled with said fixed medical substance in a specific manner to said sample;

bringing the mixture in contact with the resonance material to which said medical substance has been fixed;

making a light incident upon said resonance material;

detecting a change of the incident light or a change of a reflected light thereof when said resonance phenomenon is generated; and

recognizing an amount of medical substance contained in said sample on the basis of said change of the incident light or the reflected light.

25. A method for measuring a medical substance according to claim 24, wherein said resonance phenomenon is a surface plasmon resonance phenomenon.

26. An apparatus according to claim 16, wherein said resonance phenomenon is a surfaceplasmon resonance phenomenon.

27. An apparatus according to claim 17, wherein said resonance phenomenon is a surface plasmon resonance phenomenon.

APPENDIX II – CLAIMS

14. (as of December 10 Amendment) An apparatus for measuring a medical substance (an antigen) contained in a sample using a resonance phenomenon resonating with an evanescent wave, said apparatus, comprising:

a resonance phenomenon generating section having a resonance material; and

a detecting means for detecting a change of an incident light which is made incident upon said resonance material to generate said resonance phenomenon or a change of a reflected light thereof

wherein the medical substance (antigen) to be measured is fixed to said resonance material.

15. An apparatus according to claim 14, wherein said change to be detected by said detecting means is an incident angle of said light being made incident upon said resonance material when an intensity of the reflected light thereof is decreased.

16. An apparatus according to claim 14, wherein said change to be detected by said detecting means is a wavelength or a wave number of said reflected light when an intensity of said reflected light is decreased.

17. An apparatus according to claim 14, wherein said change to be detected by said detecting means is an intensity of said reflected light when the incident light is made incident upon said resonance material with a predetermined incident angle.

18. An apparatus according to claim 14, wherein said change to be detected by said detecting means is an incident angle of said incident light when a phase of said reflected light is varied.

19. An apparatus according to claim 14, wherein said resonance phenomenon is a surface plasmon resonance phenomenon.

20. An apparatus according to claim 19, wherein said resonance phenomenon generating section comprises a prism having a high refractive index, a thin metal film directly or indirectly formed on one of the surfaces of said prism as said resonance material, and a light source for making a light incident upon said metal film via said prism, wherein the medical substance (antigen) to be measured is fixed to a surface of said metal film.

21. An apparatus according to claim 20 further comprising a calculating means for recognizing an amount of said medical substance (antigen) contained in said sample in accordance with the change detected by said detecting means.

22. A medical substance sensor for use in an apparatus for measuring a medical substance (an antigen) contained in a sample using a resonance phenomenon resonating with an evanescent wave comprising a resonance material where the resonance phenomenon is caused to resonate with an evanescent wave, wherein the medical substance (antigen) to be measured is fixed to said resonance material.

23. A medical substance sensor according to claim 22 further comprising a prism having a high refractive index, a thin metal film which is directly or indirectly formed on one of the surfaces of said prism as said resonance material, wherein the medical substance (antigen) to be measured is fixed to a surface of said metal film.

24. A method for measuring a medical substance (an antigen) contained in a sample using a resonance phenomenon resonating with an evanescent wave, said method comprising the steps of:

fixing a medical substance (an antigen) to be measured to a resonance material wherein the resonance phenomenon is caused to resonate with an evanescent wave;

mixing an antibody with said sample wherein the antibody is coupled with said medical substance (antigen) in a specific manner;

bringing a mixture of said antibody and said sample in contact with the resonance material to which said medical substance (antigen) has been fixed;

making a light incident upon said resonance material;

detecting a change of the incident light or a change of a reflected light thereof when said resonance phenomenon is generated; and

recognizing an amount of said medical substance (antigen) contained in said sample on the basis of said change of the incident light or the reflected light.

25. A method for measuring a medical substance according to claim 24, wherein said resonance phenomenon is a surface plasmon resonance phenomenon.

26. An apparatus according to claim 16, wherein said resonance phenomenon is a surface plasmon resonance phenomenon.

27. An apparatus according to claim 17, wherein said resonance phenomenon is a surface plasmon resonance phenomenon.

United States Patent [19]

Batchelder et al.

[11] **Patent Number: 4,844,613**

[45] **Date of Patent: Jul. 4, 1989**

[54] **OPTICAL SURFACE PLASMON SENSOR DEVICE**

[75] Inventors: **David N. Batchelder**, London; **Jolyon P. Willson**, Duxford, both of Great Britain

[73] Assignee: **STC PLC**, London, England

[21] Appl. No.: **115,766**

[22] Filed: **Nov. 2, 1987**

[30] **Foreign Application Priority Data**

Nov. 3, 1986 [GB] United Kingdom 8626221

[51] **Int. Cl.**[4] **G01N 21/55**; G01N 21/63

[52] **U.S. Cl.** **356/318**; 356/445

[58] **Field of Search** 356/311, 317, 318, 445

[56] **References Cited**

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4,681,451 7/1987 Guerra et al. 356/373

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2173895 10/1986 United Kingdom 356/318

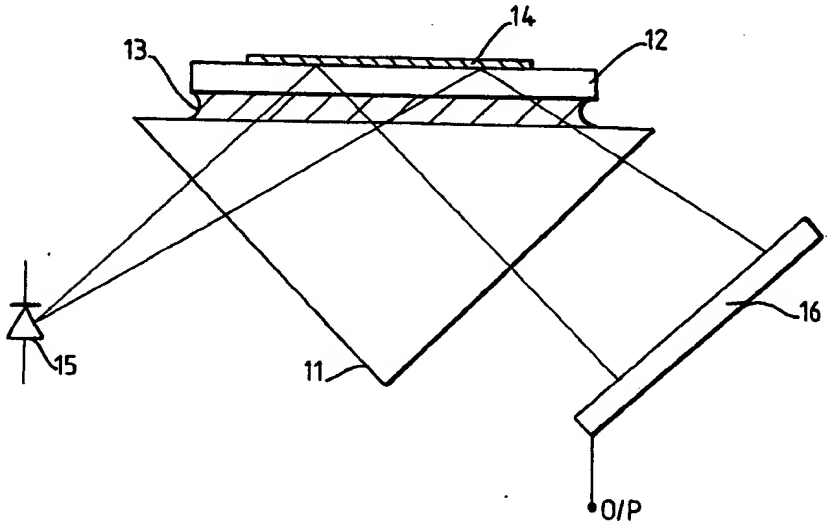
Primary Examiner—Vincent P. McGraw

Attorney, Agent, or Firm—Lee & Smith

[57] **ABSTRACT**

An optical sensor device uses surface plasmon resonance to detect the presence of a specific material. A transparent body (**12**) is coated with a thin gold film (**14**) which film may be coated e.g. with an antibody material. The arrangement is illuminated with a divergent light beam and light internally reflected from the gold film is detected by a photodiode array (**16**). The dielectric conditions adjacent the gold film determine the position of the surface resonance angle, this being indicated by a dark area on the detector array.

7 Claims, 2 Drawing Sheets



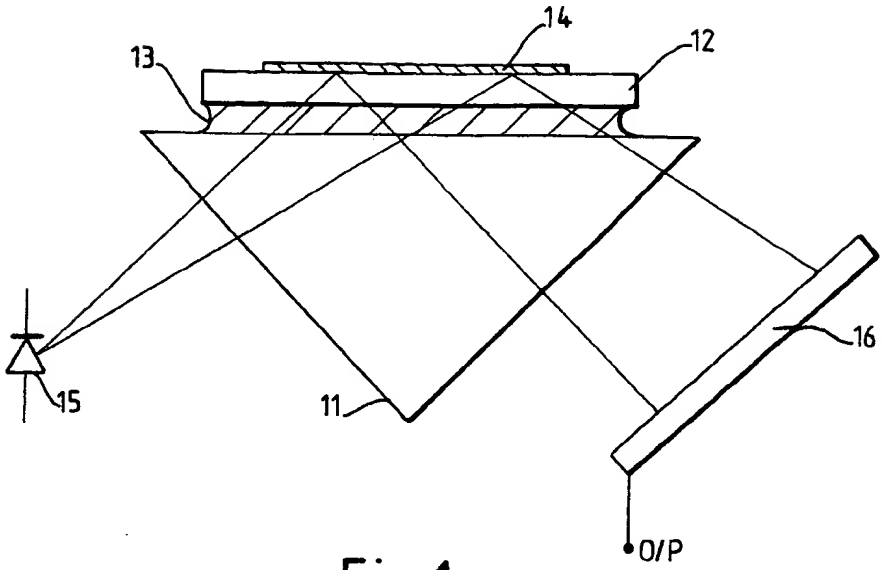


Fig.1

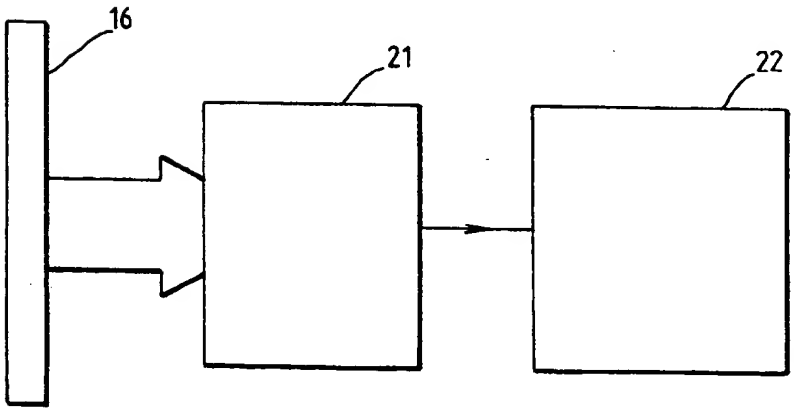


Fig.2

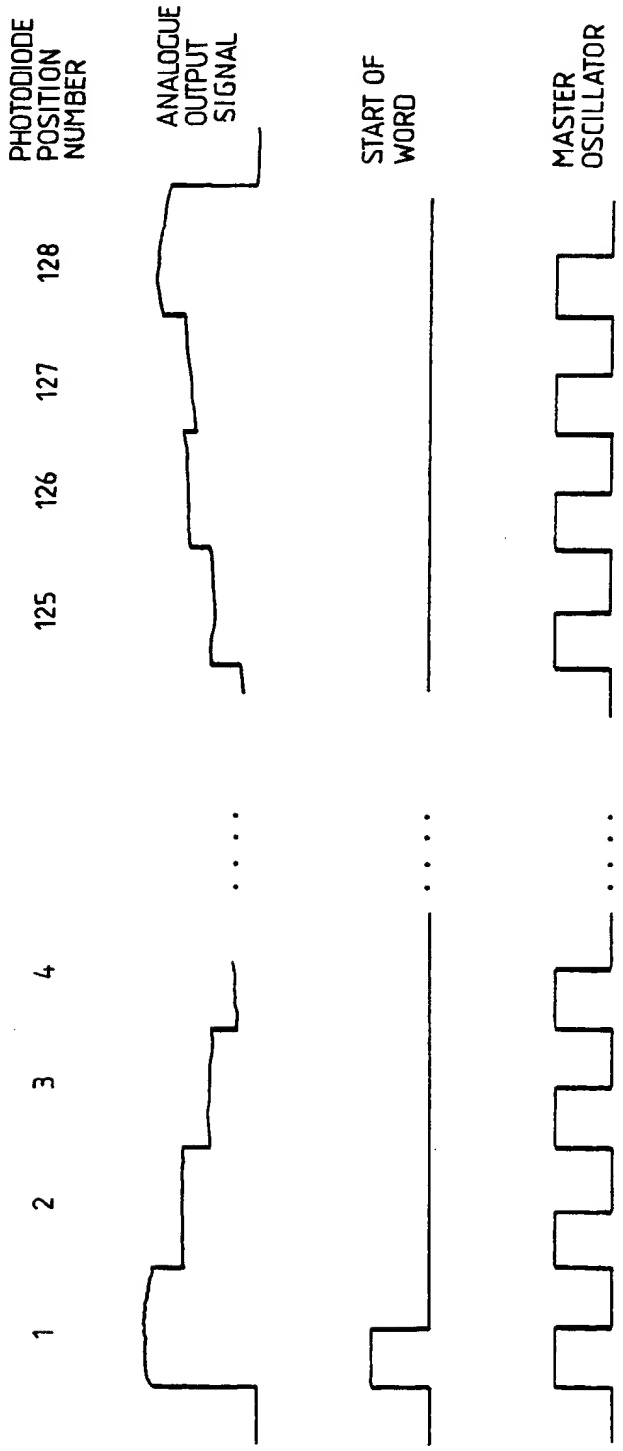

PHOTODIODE POSITION NUMBER
ANALOGUE OUTPUT SIGNAL
START OF WORD
MASTER OSCILLATOR
1
2
3
4
125
126
127
128

Fig. 3

OPTICAL SURFACE PLASMON SENSOR DEVICE

This invention relates to optical sensors, e.g. for chemical, biochemical or biological analysis.

BACKGROUND OF THE INVENTION

Surface plasmon resonance is an optical surface phenomenon that has recently been employed in the construction of sensors. A surface plasmon is a surface charge density wave at a metal surface. A physical description of the phenomenon is given by H. Raether in Phys. Thin Films, 1977, 74 pp 237–244. The resonance can be observed when the evanescent field of a p-polarised light beam, totally internally reflected from a dielectric interface, interacts with a thin metal film applied to the interface. Typically the interface comprises a smooth surface of a transparent, e.g. glass, body. Light reflected internally from the surface exhibits a minimum intensity for a particular (resonant) angle of incidence, this angle being determined by the dielectric conditions adjacent the metal film and the properties of the metal film itself.

Plasmon resonance is observed when the component of the evanescent field wave vector parallel to the metal/dielectric interface (K_x) is equal to the surface plasmon wave vector (K_{sp}) as given by the following equation:

$$K_x = \frac{W}{C}\sqrt{\epsilon_1}\sin\theta = K_{sp} = \frac{W}{C}\left(\frac{1}{\epsilon_2}+\frac{1}{\epsilon_m}\right)^{-\frac{1}{2}}$$

where W is the optical frequency, C the free space velocity of light and ϵ_m is the real part of the dielectric constant of the metal. ϵ_1 is the dielectric constant of the prism and ϵ_2 is the dielectric constant of a dielectric applied to the metal. θ is the angle of incidence of the optical beam at the metal/dielectric interface. Thus the value of the wave vector at resonance is a function of both dielectric constants, the optical wavelength and of the metal.

In a prior art sensor using this phenomenon, a metal film is applied to one surface of a glass prism. Such a device is described in Electronics Letters, 8th Nov. 1984, 20, No. 23, pp 968 to 970. In this device the resonant angle is determined by varying the angle of incidence of light directed through the prism to the surface and measuring the intensity of the reflected light. Such an arrangement requires a high degree of precision in the manufacture of its optical moving parts to provide accurate measurement.

The object of the present invention is to minimise or to overcome this disadvantage.

SUMMARY OF THE INVENTION

According to the invention there is provided an optical sensor device, the device including a transparent body having a major surface, a thin conductive film supported on said surface, means for directing a divergent light beam through the body towards said surface so as to excite surface plasmons in the conductive film, and means for detecting the pattern of light reflected internally from the major surface so as, in use, to determine the angle or angles of incidence at which plasmon resonance occurs.

As there are no moving parts the problem of high precision manufacture is alleviated. Typically the reflected light pattern is detected via a photodetector array e.g. of the type employed in a television camera tube. Typically the transparent body is formed of glass on a plastics material.

An embodiment of the invention will now be described with reference to the accompanying drawings in which:

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a sectional schematic view of the surface wave plasmon sensor device;

FIG. 2 shows a data processing system for use with the sensor of FIG. 1, and

FIG. 3 illustrates data format waveforms used in the system.

DESCRIPTION OF PREFERRED EMBODIMENT

Referring to FIG. 1, the sensor device includes a transparent prism 11, e.g. of equilateral triangular cross-section, on the surface of which is mounted a glass microscope slide or cover slip 12. The airgap between the slide 12 and the prism 11 is filled with a quantity of index matching fluid 13. Where the prism 11 is of glass we prefer to employ glycerol (n=1.47) as the index matching fluid. The upper surface of the slide 12 is coated with a thin conductive layer 14 e.g. gold, typically 400 to 700 A (40 to 70 nm) in thickness. This layer 14 provides the conductive surface layer in which, in use, surface plasmons are excited.

Light is directed to the prism assembly from a light source 15 comprising e.g. a light emitting diode. Advantageously the light source 15 has an output wavelength in the range 500 nm to 900 nm. The light from the source 15 is incident on the prism in the form of a divergent beam. This beam, after refraction at the glass/-metal interface passes back through the prism 11 to a detector array 16. The image 'seen' by the array comprises a substantially uniformly illuminated area with a dark band corresponding to the angle or angles at which plasmon resonance reduces the intensity of reflected light. The position of the absorption band may be determined by a microprocessor (not shown) coupled to the detection array 16.

The angular position of the plasmon resonance is a function of the dielectric constant of a medium in contact with the gold film 14. As the electric field associated with the plasmon decays exponentially into the medium, the device is sensitive only to changes close to the gold surface, typically within 1000 Augstroms. In general the device is used in chemical or biological applications to detect species present in aqueous solutions, e.g. blood serum, whose refractive index is 1.33 to 1.35. For biosensing applications the gold film 14 may be coated with a layer, typically 50 to 100 A thick, of an antibody whose refractive index is 1.5 to 1.6. As the refractive index of the antibody layer differs from that of the adjacent solution, a change in the antibody layer thickness emitting from bonding sheets of a corresponding antigen causes a corresponding change in the plasmon resonance angle. Typically the sensitivity of the device is such that a change of 1A in the antibody layer thickness causes a change of 0.01° in the resonance angle for a source wavelength of 820 nm.

The sensitivity of the device may be improved by the use of a light source of short wavelength so that the plasmon penetration depth is then smaller. For example, a source wavelength of 560 nm gives a sensitivity of

about 0.1°/A. However, it should be noted that, if lower sensitivity can be toleratcd, working at longer wavelengths is to be preferred as, at such wavelength, the spectral line width (10–50 nm) of LED sources does not unduly broaden the angular width of the resonance. At short wavelengths this effect can be mitigated by the use of a narrow band filter or by the use of a gas laser as the light source. For example, a helium/neon gas laser has suitable output wavelength at 543 nm and 594 nm.

In an alternative arrangement a pair of similar light sources may be employed. One light source is used to provide sensing whilst the other provides a reference channel to compensate e.g. for non-specific binding effects. The light sources and sample sites are arranged so that the reflected divergent beams are both received by the photodiode array. By selectively enabling the light sources the plasmon resonance angle can be accurately measured for two sample sites only one of which is coated with the antibody. The difference in plasmon resonance angle is then due solely to specific binding effects. For a more accurate cancellation of non-specific binding, the second site can be coated with a different antibody with similar dielectric characteristics, or a deposited dielectric film.

The accuracy of measurement of the sensor system of FIG. 1 may be enhanced by the use of a data aquisition arrangement. Such an arrangement is shown in FIG. 2 of the accompanying drawings. The operation of this data aquisition arrangement is described below with reference to a photodiode arrangement having 128 elements, but it will be clear that this description is given by way of example only, and that alternative arrangements may be employed.

The outputs of the photodiodes of the array are fed via a data aquisition module 21 to a computer 22. The computer determines the position of minimum light intensity, i.e. the plasma resonance angle, by a curve fitting process which identifies this minimum to a high degree of accuracy.

The data aquisition module 21 provides the computer 22 with the following signals which are illustrated in FIG. 3 of the accompanying drawing:

(i) An analogue signal, which consists of a series of words where each word comprises 128 pulses and the height of each pulse corresponds to the intensity of the light falling on the corresponding photodiode.

(ii) A master oscillator signal which goes high at the beginning of each pulse in the analogue output signal.

(iii) A start of word signal which goes high at the beginning of each word of the analogue output signal. The master oscillator and therefore also the analogue output signal may have a frequency of about 10 kHz.

Processing of the input data is effected by the computer in a two stage process. Firstly, each input word is evaluated to determine the position at which the minimum light intensity occurs. Data corresponding to the outputs of the 40 photo detections measurement to this minimum position is then stored for analysis in the second stage of the process.

The second stage involves fitting of a polynomial, e.g. a fourth order polynomial, to the 40 readings obtained from the previous stage. The method used is to minimise the squares of the differences between the stored values and the values calculated for a general fourth order polynomial. Having obtained expressions for the spaces of the differences, these are used to form a system of linear homogeneous equations. This system of equations is solved by matrix inversion to give the desired polynomial. The characteristics of this polynomial are then evaluated to determine its turning points and thus to determine the precise position of the minimum value.

It is preferred that correction factors be applied to each element of the 128 element word to compensate for differences in the photo detector elements of the array.

It is known that each element of the array has a different dark-current and that each element becomes saturated at a different level of light intensity, i.e., the relationship between voltage output and light intensity is different for each element of the array, and they differ by at least two parameters. It is assumed that the relationship is linear and thus has exactly two parameters which can be calculated for each photodiode by taking two calibration readings. It is also assumed that for the Ith photodiode there exist numbers offset (I) and linmult (I) such that:

$$V_I = (L \times \text{limult } [I] + \text{offset } (I))$$

where L=Light intensity on Ith photodiode and V_I=-Voltage od Ith pulse in analogue output signal word.

First, there is no light falling on the array, ten "words" are read from the photodiode array, and for each I an average height of the Ith pulse is calculated. These are the values of offset (I). Then when each photodiode in the array has the same light intensity falling on it, ten more "words" again are read fron the photodiode array and an average output for each array element is again calculated. An average of all the heights of all the pulses is also calculated (i.e., the average of 10×128 numbers) and this is assumed to be the true light intensity (i.e., L is the equation above). Thus for each I linmult (I) can be calculated using the formula.

$$\text{linmult } (I) = \frac{v - \text{offset } (I)}{L}$$

To illustrate the technique, a clear microscope slide was coated with a 45nm thick layer of gold. The gold surface was coated with a monolayer of thyroid stimulating hormone antibody. Half the slide area was then coated with a monolayer of thyroid stimulating hormone. The slide was mounted on a glass prism and covered with a water film. The arrangement was illuminate using a Honeywell (registered Trade Mark) Sweetspot LED source. The difference in plasmon resonance angle determined by measurements of the two halves of the slide was found to be 0.07°. This illustrates the facility of detection of biochemical materials using the arrangement described herein.

Although the sensor has been described with particular reference to biological or biochemical applications it can of course also be employed as a sensor in purely chemical applications.

We claim:

1. An optical sensor device, including a transparent body having a major surface on which a thin electrically conductive film is disposed, a light source fixed in position relative to the body and arranged to direct a divergent monochromatic light beam through the body to the surface whereby to achieve total internal reflection of the light from that surface and to excite surface plasmons in the conductive film, and an array of photodetectors arranged so as to detect the pattern of light reflected internally from the major surface whereby to

determine the angle of incidence at that surface at which plasmon resonance occurs.

2. A sensor device as claimed in claim 1, wherein the conductive film comprises gold.

3. A sensor device as claimed in claim 2, wherein the conductive film is coated with a layer of an antibody.

4. A sensor device as claimed in claim 3, wherein the transparent body is formed of glass or a plastics material.

5. A sensor device as claimed in claim 4, wherein said transparent body comprises a laminar body supported on and in optical contact with a further transparent body.

6. A sensor device as claimed in claim 1, and incorporating a further reference light source.

7. An optical sensor arrangement, including a transparent body having a major surface on which a thin conductive film is disposed, means for directing a divergent monochromatic light beam through the transparent body towards said surface so as to excite surface plasmons in the conductive film, an array of photodetectors arranged so as to receive light reflected internally at a range of angles from the major surface, means for evaluating the intensity of light received from each photodetector, and means for calculating a polynomial corresponding to said light intensities whereby to determine the angle of reflection for which a minimum light intensity indicative of plasmon resonance is obtained.

* * * * *

United States Patent [19]

Finlan et al.

[11] **Patent Number: 4,997,278**

[45] **Date of Patent: Mar. 5, 1991**

[54] **BIOLOGICAL SENSORS**

[75] Inventors: **Martin F. Finlan**, Aylesbury; **John E. M. Midgley**; **Stephen A. Charles**, both of Little Chalfont; **James C. Irlam**, Staines, all of England

[73] Assignee: **Amersham International PLC**, Buckinghamshire, England

[21] Appl. No.: **232,650**

[22] Filed: **Aug. 16, 1988**

[30] **Foreign Application Priority Data**

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Sep. 4, 1987 [GB] United Kingdom 8720854

[51] **Int. Cl.**[5] **G01N 21/41**; G01N 21/55; G01J 3/30

[52] **U.S. Cl.** **356/128**; 356/318; 356/445

[58] **Field of Search** 356/127-129, 356/132, 135, 136, 318, 317, 311, 445

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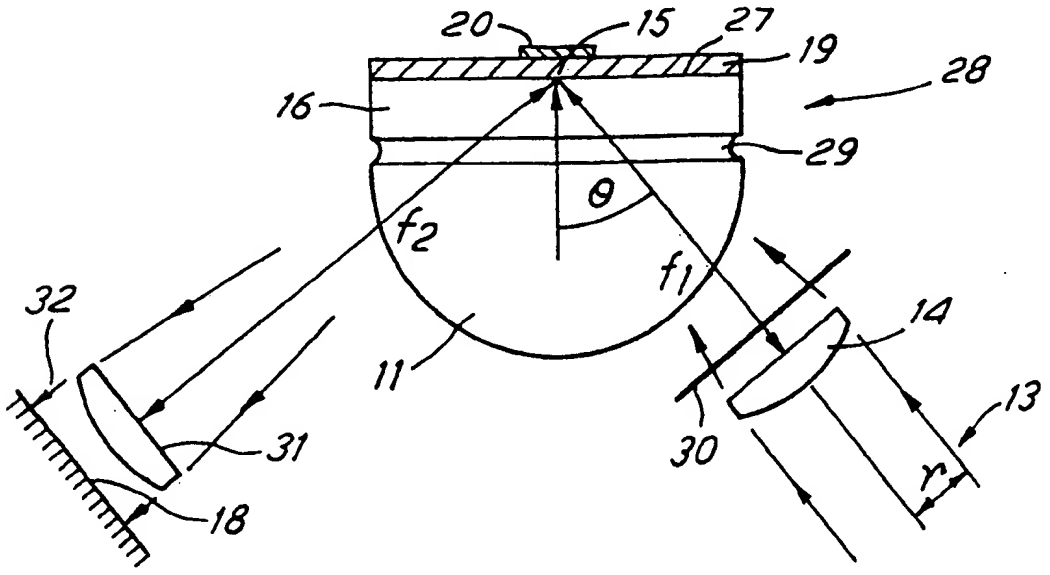
Primary Examiner—Richard A. Rosenberger
Assistant Examiner—Hoa Pham
Attorney, Agent, or Firm—Wenderoth, Lind & Ponack

[57] **ABSTRACT**

A sensor uses the principle of surface plasmon resonance (SPR) to monitor the progress of the reaction between a sample and a sensitive layer (for example an antibody layer). The layer is applied to the rear surface of a metallic film formed on the surface of an optically transmissive component in the form of a hemicylindrical lens and slide. Collimated light from a source is applied via a lens which focuses the incoming beam to a focus at a point to form a fan-shaped spread of light incident at the point. The light is internally reflected at the point, and emerges from the component to be applied to a dectector array which latter is electronically scanned. The angle of incidence of the light at the point is such as to span that angle which gives rise to surface plasmon resonance, together with a range of angles thereabout so that the progress of the resonant condition, as the reaction between the sample and the sensitive layer proceeds, can be monitored.

14 Claims, 3 Drawing Sheets



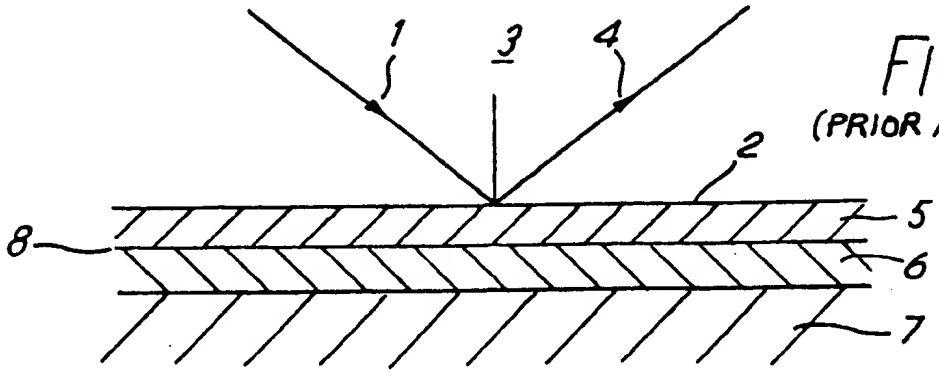


FIG. 1
(PRIOR ART)

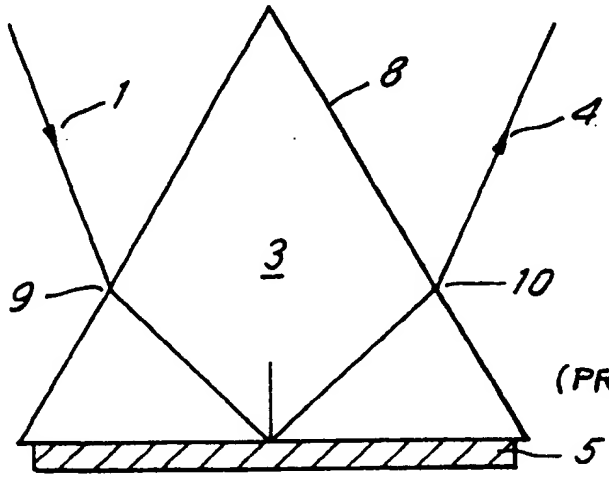


FIG. 2
(PRIOR ART)

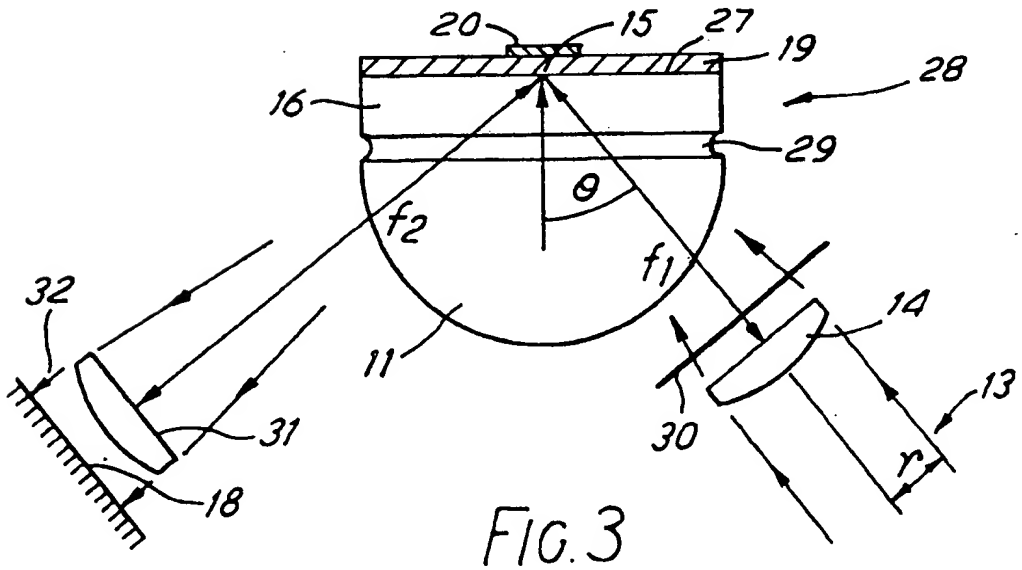


FIG. 3

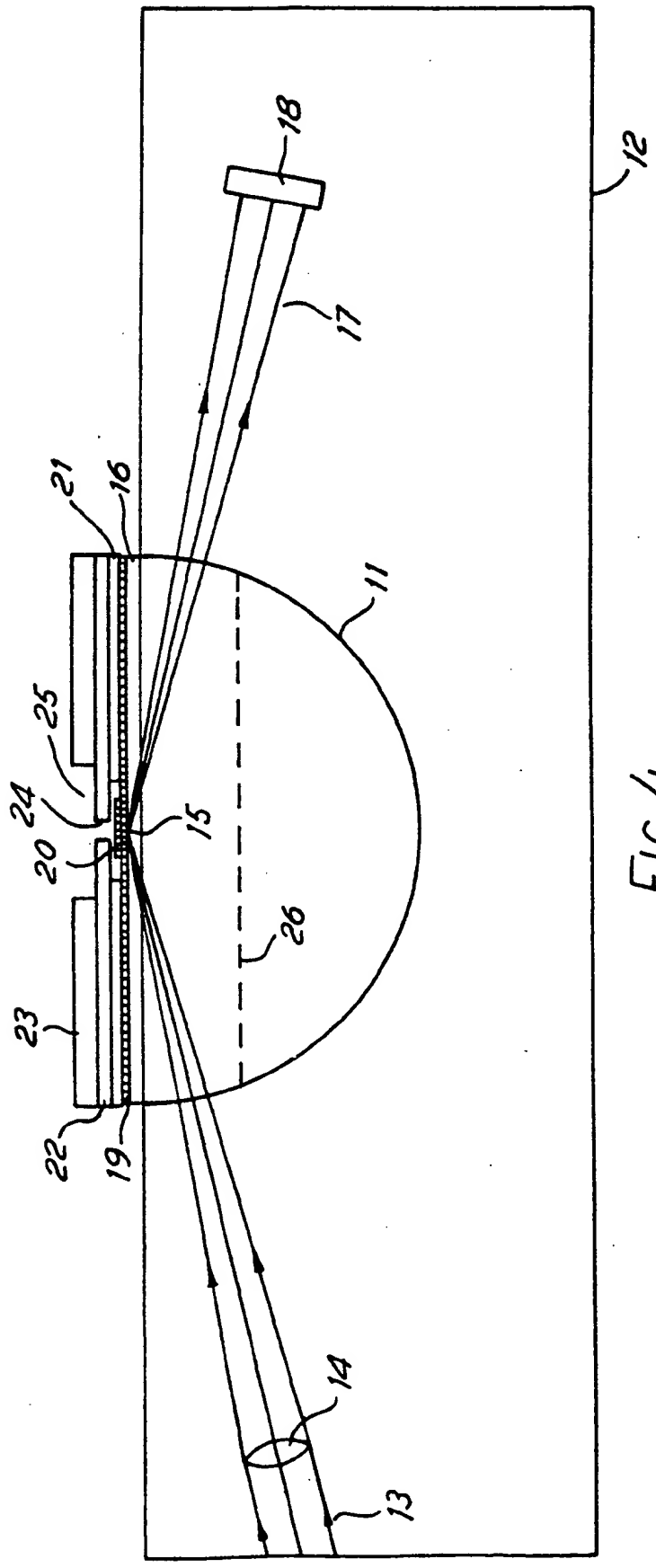


FIG. 4

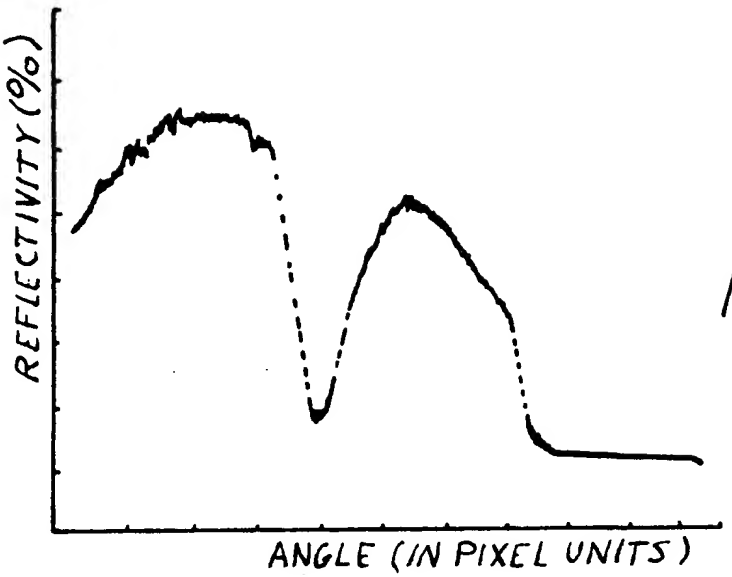

REFLECTIVITY (%)
ANGLE (IN PIXEL UNITS)

FIG. 5(a)

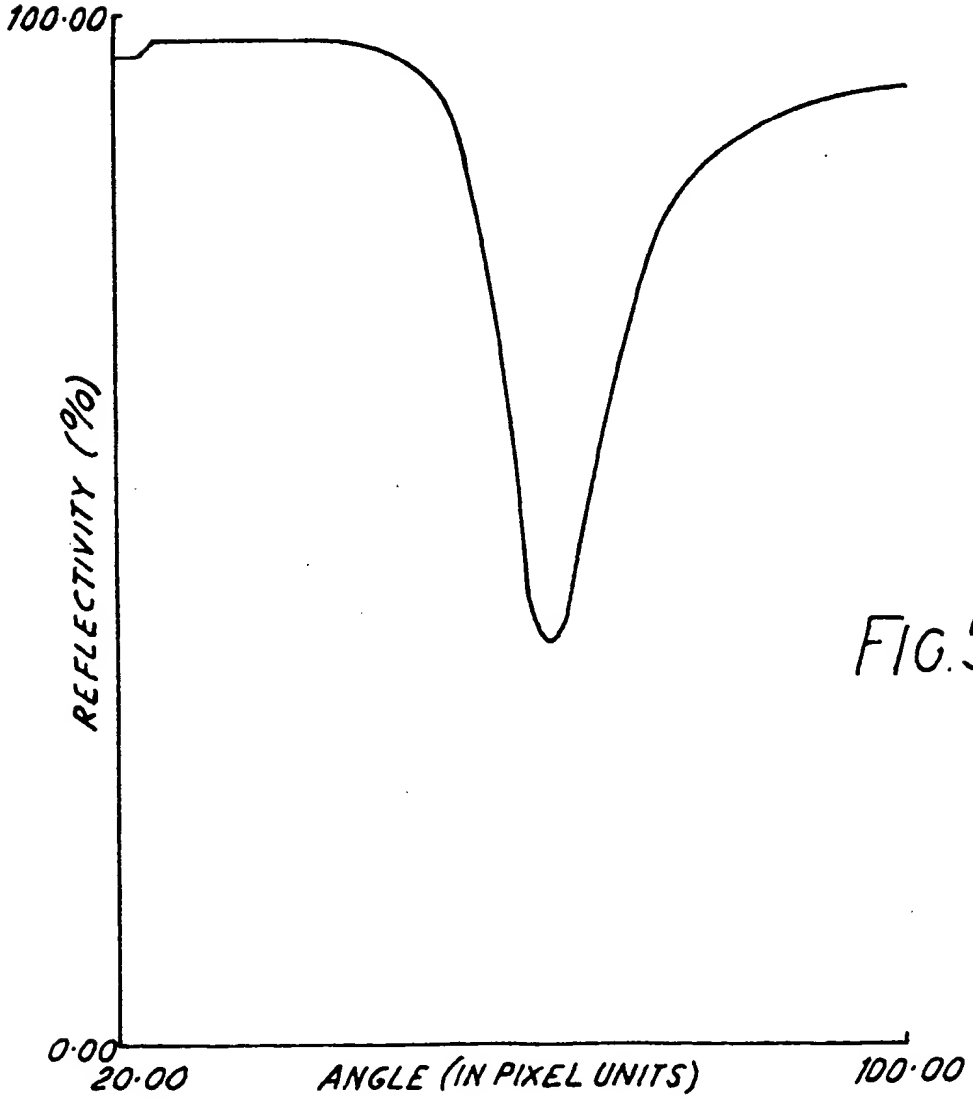

100·00
REFLECTIVITY (%)
0·00
20·00
ANGLE (IN PIXEL UNITS)
100·00

FIG. 5(b)

BIOLOGICAL SENSORS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to sensors for use in biological, biochemical and chemical testing and in particular to immunosensors used to monitor the interaction of antibodies with their corresponding antigens.

2. Description of the Related Art

When antibodies are immobilized on a surface, the properties of the surface change when a solution containing a corresponding antigen is brought into contact with the surface to thus allow the antigen to bind with the antibody. In particular, the change in the optical properties of the surface can be monitored with suitable apparatus.

The phenomenon of surface plasmon resonance (SPR) can be used to detect minute changes in the refractive index of the surface as the reaction between the antigen and the antibody proceeds. Surface plasmon resonance is the oscillation of the plasma of free electrons which exists at a metal boundary. These oscillations are affected by the refractive index of the material adjacent the metal surface and it is this that forms the basis of the sensor mechanism. Surface plasmon resonance may be achieved by using the evanescent wave which is generated when a p-polarized light beam is totally internally reflected at the boundary of a medium, e.g. glass, which has a high dielectric constant. A paper describing the technique has been published under the title "Surface plasmon resonance for gas detection and biosensing" by Lieberg, Nylander and Lundstrom in Sensors and Actuators, Vol. 4, page 299. Illustrated in FIG. 1 of the accompanying drawings is a diagram of the eqipment described in this paper. A beam 1 of light is applied from a laser source (not shown) onto an internal surface 2 of a glass body 3. A detector (not shown) monitors the internally reflected beam 4. Applied to the external surface 2 of glass body 3 is a thin film 5 of metal, for example gold or silver, and applied to the film 5 is a further thin film 6 of organic material containing antibodies. A sample 7 containing antigen is brought into contact with the antibody film 6 to thus cause a reaction between the antigen and the antibody. If binding occurs, the refractive index of the layer 6 will change owing to the size of the antibody molecules and this change can be detected and measured using the surface plasmon resonance technique, as will now be explained.

Surface plasmon resonance can be experimentally observed, in the arrangement of FIG. 1, by varying the angle of the incident beam 1 and monitoring the intensity of the internally reflected beam 4. At a certain angle of incidence the parallel component of the light momentum will match with the dispersion for surface plasmons at the opposite surface 8 of the metal film. Provided that the thickness of metal film 5 is chosen correctly, there will be an electromagnetic coupling between the glass/-metal interface at surface 2 and the metal/antibody interface at surface 8 as a result of surface plasmon resonance, and thus an attenuation in the reflected beam 4 at that particular angle of incidence. Thus, as the angle of incidence of beam 1 is varied, surface plasmon resonance is observed as a sharp dip in the intensity of the internally reflected beam 4 at a particular angle of incidence. The angle of incidence at which resonance occurs is affected by the refractive index of the material

against the metal film 5—i.e. the antibody layer 6—and the angle of incidence corresponding to resonance is thus a direct measure of the state of the reaction between the antibody and their antigen. Increased sensitivity can be obtained by choosing an angle of incidence half way down the reflectance dip curve, where the response is substantially linear, at the beginning of the antibody/antigen reaction, and then maintaining that angle of incidence fixed and observing changes in the intensity of the reflected beam 4 with time.

Known systems of the type described with reference to FIG. 1 utilize a prism as the glass body 3. A diagram showing this arrangement is given in FIG. 2 which is simply an experimental set up intended to demonstrate surface plasmon resonance. The prism is shown under reference 8 and has applied to its undersurface a thin film 5 of metal. Light 1 from a laser source (not shown) is incident on the prism where it is refracted at point 9 before entering the prism. The internally reflected beam 4 is likewise refracted (at point 10) upon exiting from the prism.

One problem with the known SPR systems is the slowness of operation relative to changes in the refractive index of the antibody layer. Another problem, particularly related to the use of the prism shown in FIG. 2, is that, as the angle of incidence is changed, either by moving the source, or rotating the prism, or both, the point on surface 2 at which the incoming beam is incident moves. Because of inevitable variations in the metal film 5 and the coating 6 of antibody, the angle of incidence which results in resonance changes as this movement occurs, which in turn introduces a further variable factor into the measurement and thus makes comparisons between the initial, unbound, state and the bound state of the antibody layer 6 less accurate.

SUMMARY OF THE INVENTION

In the present invention, the speed of response is improved by providing that the incoming beam of radiation which is internally reflected at the glass/metal interface takes the form of a fan-shaped beam of electromagnetic radiation, usually in the visible or near-visible region. In this way, the progress of the resonant condition, as the reaction between the sample and the antibody layer proceeds, can be monitored. In one example, this can be achieved by taking a "solid" input beam from a source of electromagnetic radiation, and bringing it (the beam) to a focus at the point of incidence of the beam on the glass/metal interface. The input beam thus becomes equivalent to several beams incident upon the glass/metal interface over a range of angles. The equipment can be chosen so that the range of angles spans the angle of dip corresponding to surface plasmon resonance together with a range of angles thereabout. The corresponding internally reflected beam is likewise effectively several beams and may be monitored by a large area detector, or by an array of angularly spaced detectors positioned to collect the several emergent beams. Thus the detectors can encode the information from the whole of the dip within milliseconds. In this way, the progress of the resonant condition, as the reaction between the sample and the antibody layer proceeds, can be monitored.

The use of a fan-shaped beam highlights the problems of the prism (see above) and, in order to avoid these, it is provided that the surface of the transparent, usually glass, body onto which the incoming light is incident is

a curved, preferably circular, surface and is arranged, with respect to the input beam of electromagnetic radiation, such that the beam enters orthogonally to the tangent to the surface at the point of entry. Preferably likewise, that surface from which the internally reflected beam emerges is a curved, preferably circular, surface.

In a first embodiment of the invention, the transparent body takes the form of a glass hemispherical body whose flat surface is covered with a thin metal film and a sensitive overlayer in the manner described above. The source of input electromagnetic radiation, for example a light source, is arranged so that the input beam enters the hemispherical body orthogonally to the tangent at the point of incidence, and thus the beam passes through unrefracted and is incident at the center of the circular flat surface. The point of incidence on the flat surface is thus the same for all parts of the fan-shaped beam.

Shapes other than hemispherical can be used; for example semicylindrical, which gives a line incidence, rather than a point, or truncated hemispherical or hemicylindrical in which the top is cut off—i.e. to form a body having two flat, probably parallel, surfaces with arcuate sides joining the surfaces.

The fan-shaped beam may be constrained to be substantially planar by being projected through a slit lying in a plane passing through the point of incidence and oriented vertically to that of the glass/metal interface. Alternatively, the expression "fan-shaped" may refer to a shape of a section of the input beam, and the beam itself may extend in other planes—for example wedge-shaped (giving a line of incidence), or conical shaped.

Although the layer applied to the metal film is described herein as an antibody layer for use in immunoassays, it will be seen that any sensitive layer whose refractive index changes upon an event occurring can be used to thus provide a sensitive detector having a wide variety of applications in the fields of biology, biochemistry and chemistry. As an example, the sensitive layer could be a DNA or RNA probe which would, during the test, bind with its complement in solution as represented by the sample to be tested.

The metal film material is commonly silver or gold, usually applied by evaporation. The film needs to be as uniform as possible in order to cater for minute movement in the point of incidence of the incoming beam. It is assumed that a structured metal film will give the best resonance and there are various ways in which the glass body can be pretreated to improve the performance of the metal film and in particular to control the natural tendency of such films to form discontinuous islands:

1. Immersion in molten metal nitrates and other molten salts. This has the effect of introducing ions into the surface in a manner which can be structured and which can act as foci for island formation.

2. Ion bombardment of cold or hot glass to introduce nucleating sites. The removal of the more mobile ions has been demonstrated to reduce the thickness at which the evaporated film becomes continuous.

3. Electroless plating or electroplating over lightly evaporated films (0 to 100 angstroms thick). Electroless plated films survive to a greater thickness than evaporated films and could form more stable nuclei for subsequent coating.

4. Evaporating on to electroless plated films. The electroless plated films have a stronger tendency to an island structure and to bigger islands with greater spacing than evaporating films. This could be of advantage in tuning to light of a prescribed wavelength.

Coating performance can also be improved by:

1. Controlling the glass surface temperature during coating. Using a higher temperature substrate increases the islands' size and the spacing between them and conversely.

2. Evaporating in the presence of a magnetic or electrostatic field or electron emission device to control the ion content of the vapor stream. The state of charge of the substrate is know to affect the island structure.

3. Controlling the angle of incidence of the evaporated vapor stream relative to the glass surface. The mobility of the evaporated atoms and hence their ability to form bigger islands is greater when the momentum of the atoms relative to the glass surface is increased.

BRIEF DESCRIPTION OF THE DRAWINGS

In order that the invention may be better understood, some embodiments thereof will now be described by way of example only and with reference to the accompanying drawings in which:

FIGS. **1** and **2** are diagrams of known experimental arrangements for demonstrating the surface plasmon resonance effect;

FIG. **3** shows, in schematic outline, a cross-sectional view of a sensor in accordance with one example of the invention;

FIG. **4** is a diagrammatic side view of another example of a sensor according to the present invention; and

FIGS. **5**(*a*) and **5**(*b*) illustrate the performance of which an arrangement in accordance with the invention is capable.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring now to FIG. **3**, a collimated beam **13** of electromagnetic radiation of width 2 r from a source, which is not shown but may conveniently comprise a laser diode collimator pen such as that manufactured under the model number TXCK 1200 by Telefunken Electronic, is incident upon a hemi-cylindrical focussing lens **14** of focal length f_1, which causes the light to converge to a point **15** on an interface **27** between an optically transmissive component, generally shown at **28**, and a reflective layer **19** in the form of a metallic coating. The optical component is, in this example, made up of a glass support plate or slide **16** having a first surface (upon which the reflective layer is coated,) and a hemispherical lens **11** having a second, curved, spherical surface with its center of curvature located at the point **15**. A suitable index matching fluid is provided, as shown at **29**, between the facing surfaces of plate **16** and lens **11** and the arrangement is such that all light rays in the convergent beam which emerges from lens **14** travel radially of the optically transmissive component **28** and thus undergo no refraction and are focussed centrally on the point **15**. A slit **30** constrains the convergent beam to a substantial planar fan shape, so that only a small area of reflective layer **19** is illuminated to reduce any effects due to non-uniformity of the metal coating.

The light internally reflected from point **15** travels as a divergent, planar, fan-shaped beam back out of the component **28**, through a third surface thereof contiguous to the second surface as shown in FIG. **3**, and is incident upon a focussing lens **31** which transmits such light as a light beam **32** which is substantially parallel-sided, or at least of reduced divergence compared to the

fan-shaped beam of light emergent from component **28**. Beam **32** is arranged to be incident upon a detector **18**, for example an array of photo-sensitive detectors, and in particular an angular array, and it will be appreciated that the main purpose of lens **31** is to reduce stray reflections in the array **18** ensuring that beam **32** is normal to its surface. If, however, the stray reflections are not of significance or if the array **18** can be conveniently placed close to the exit surface of component **28** (possibly even attached to or deposited on that surface) lens **31** is not required.

The array of detectors is arranged to generate electrical signals indicative of the variation of intensity of light with position across the beam **32**; the SPR effect dictating that strong absorption will occur at a particular angle as determined by material in the fluid to which the reflective layer **19** is exposed. These electrical signals are sampled and digitized and fed to a suitable analyzing arrangement which may include a microprocessor or larger computer.

It can be desirable, in the interests of minimizing the disturbing effects of extraneous light without having to resort to the expense and inconvenience of shrouding the entire arrangement, or at least the components **5** and **28**, to arrange that a characteristic modulation is impressed upon the light and that the detectors and/or the processing circuits are "tuned" to respond preferentially to such modulation.

A second embodiment of the invention will now be described by reference to FIG. **4**. Referring to FIG. **4**, the apparatus comprises a hemispherical body **11** made of transparent material such as glass or quartz housed within a casing **12**. A source (not shown) of electromagnetic radiation produces a collimated input beam **13** of electromagnetic radiation. The frequency of the radiation must be such as to result in the generation of surface plasmon waves and in practice will be within or near the visible region. Suitable sources include a helium neon laser or an infrared diode laser, but an ordinary light source, with suitable filters and collimators, could be used.

A lens **14** is used to bring the parallel input beam **13** to a focus at a point **15** spaced just above the center of the circular flat surface of the hemispherical body **11**. The point **15** lies on the surface first surface of a slide **16** made of transparent material such as glass whose refractive index is equal or close to that of the hemispherical body **11**. The arrangement is such that the point **15** lies at the approximate center of curvature of the curved spherical, surface second surface of the hemispherical body.

Radiation which is internally reflected at point **15** passes, through a third surface contiguous to said second surface, out of the hemispherical body in the form of a divergent beam **17** and passes into a radiation sensitive detector **18** which gives an electrical output signal for analysis by external circuitry (not shown) in the manner described above. The detector may, for example, be a diode array, or a charge couple device or similar imaging device.

In a practical realization of the apparatus, the metal film layer, shown under reference **19**, is applied to the surface of the aforementioned slide **16**. The point **15** to which the input beam is focussed thus lies on the interface between the metal film and the slide **16**. Applied to the surface of the metal film is a sensitive layer **20** whose refractive index changes as the test progresses. The sensitive layer may, for example, be an antibody layer. The sensitive layer **20** is restricted to a relatively small active zone about the point **15** and within a central hole provided in a circular disc **21** of absorbent material. Overlying disc **21** are two further discs **22**, **23** of non-absorbent material. A central aperture in upper disc **23** defines a well **25** into which a sample to be tested is placed. A central aperture **24** in disc **22** is of a size to cause liquid in well **25** to travel by capillary action into the active zone above layer **20**. The thickness of disc **21** is such as to define a depth for the active zone such as to promote radially outward movement of the sample liquid emerging from aperture **24** by capillary action. The absorbent disc **21** absorbs sample which has flowed past the active zone.

The whole unit comprising slide **16**, disc **21** and discs **22** and **23** is disposable so that a fresh unit, including sensitive layer **20** can be used for each test. The slide **16** is placed upon the flat surface of the hemispherical body **11**, preferably after applying to the flat surface a thin layer of optical oil or grease to ensure good optical coupling between the hemispherical body and the slide. Optionally, the hemispherical body itself may be disposable, provided it can be produced cheaply enough, in which case there would be no need to include a separate slide **16**, and the metal film **19** can be applied direct to the hemispherical body.

In order to use the apparatus, a sample to be tested, and containing an antigen capable of binding with the antibody molecules in layer **20**, is placed in the well **25** and passes through aperture **24** by capillary action. Emerging from aperture **24**, the liquid sample commences to flow radially outwards in all directions towards the absorbent disc **21**, passing as it does so the antibody layer **20**. The sample adjacent the layer **20** is thus being constantly replenished during the course of the test, which ensures maximum sensitivity.

As the sample flows past the layer **20** any antigen within the sample capable of binding with the antibody in layer **20** will do so, thus altering the refractive index of layer **20** as the reaction proceeds. This change in refractive index is continuously monitored during the test by directing at the point **15** the focussed light beam **13**. Provided that conditions are correct—in particular the angle of incidence at the point **15** is correct—the application of beam **13** will result in the generation of a plasmon wave, thus extracting energy from the input beam and causing an attenuation in the intensity of the output beam **17** at a particular angle of incidence. The input beam is arranged such that the mid-angle of the range of angles of the input beam is approximately halfway down the reflectance dip, as described above, and the test is carried out at a constant angle of incidence, monitoring the intensity of the reflected beam above and below this mid point level. This gives a linear and highly sensitive output.

The initial reflectance dip which is chosen for setting up the angle of incidence should be the dip which results when some neutral or buffer solution is passed through the cell, or when the sample under test is passed through the cell but before any reaction thereof has taken place. In connection with the latter method, which is currently preferred, it is to be noted that, as sample begins to flow past the active zone adjacent layer **20** the refractive index does not start to change immediately due to the antibody/antigen reaction. There is thus sufficient time to take an initial reading with the unreacted sample flowing past, which reading can be utilized, using feedback circuitry to rapidly ad-

just the angle of incidence to an appropriate value halfway down the reflectance dip, so that the rest of the test can be performed at this fixed angle.

In an embodiment of the invention, the hemispherical body **11** is replaced by a semicylindrical body. In this case FIGS. **3** and **4** can be regarded as sections through a suitable apparatus, with the semicylindrical body **11** extending above and below the paper. The use of a semicylindrical body gives the possibility of a line area of resonance instead of the single point **15**, and hence a linear active zone. The aperture **24** becomes a slit, and the well **25** becomes elongate. The light source is operable to generate a "sheet" output beam which may be focused by a cylindrical lens of the type shown in FIG. **3** by reference numeral **31** onto a line extending through point **15**. The detector **18** is likewise linear in extent and is preferably composed of separate detectors or detector arrays, each arranged to monitor a specific section along the length of the line **15**.

The semicylindrical lens **11** has the advantage that it can be used to perform several tests simultaneously on a single sample. To this end, the layer **20** takes the form of a series of distinct sensitive areas, each comprising a different antibody, with each separate area being monitored by its own detector **18**. A single sample introduced into well **25** will flow through the slot **24** into the active area and will react simultaneously with the various sensitive areas, giving individual output readings which can be monitored at detectors **18**.

Although the hemispherical/semicylindrical body **11** is shown as having a complete 180° curvature, in fact it will be noted that only that part near the flat surface is used and therefore a substantial portion of the body **11** can be cut away to form a truncated hemispherical or semicylindrical body, as indicated, by way of example, by the dotted line **26** in FIG. **4**.

As will be appreciated from the foregoing, the invention enables a whole, or at least a significant part of, the spread of angles of interest to be investigated at once; the speed of investigation being limited only by the response characteristics of the detectors in the array **18** and of the associated sampling and computing circuits. This enables initial transients and other shifts which may occur during the analysis to be monitored and allowed for and also permits rapid calibratory checks to be made. Furthermore it has been found that, if each analysis, or assay, is started at a fixed value of reflectivity (as determined by a suitable output from the computing circuits) then the absolute refractive index of the fluid sample, which may well vary between samples, is unimportant. Importantly, the invention enables the desired reflectivity characteristic to be determined on a time scale so short that it is less than the time taken for the chemical bonding, necessary to SPR, to be achieved between the relevant constituent of the fluid sample and the reflective layer.

A further advantage of the invention is that it permits calibratory scans to be conducted with fluids of known SPR characteristics to generate compensating data which can be held in the computing circuits, and automatically applied as corrections if desired during clinical analysis. This compensating data can be used, for example, to allow for variations in reflectivity over the point **15**, a phenomenon which can occur particularly if the reflective layer is produced by evaporation.

FIG. **5** shows a representation of a video signal derived from the detector **18** in the arrangement of FIGS. **3** and **4**, as displayed on an oscilloscope screen. The SPR resonance can be clearly seen.

The detector is electronically scanned, typically at approximately 200 times per second, to allow the movement of the resonance to be viewed in "real-time" as biochemicals are bound to the surface of the metal coated plate **16**. The reflectivity curve in FIG. **5***a* has been modulated by the approximately Gaussian profile of the beam from the laser diode source. This profile can be removed by appropriate signal processing as shown in FIG. **5***b*, which was produced by subtraction of the fixed backround due to ambient light and division by the signal without any resonance.

We claim:

1. A sensor for use in biological, biochemical or chemical testing, said sensor comprising a block of material transparent to electromagnetic radiation, a layer of metallic material applied to at least part of a first surface of said block, a layer of sensitive material applied to the metallic layer, means for introducing onto the sensitive layer so as to react therewith a sample to be analyzed, a source of electromagnetic radiation, said radiation being directed into said transparent block in such a way as to be internally reflected off said part of said surface, means for converging said radiation onto said part of said surface in such a way that the incoming beam is a convergent fan-shaped beam and spans a range of angles of incidence including that which causes surface plasmon resonance to occur, the characteristics of which resonance are dependent upon the reaction between the sample and the sensitive layer, and detector means positioned to receive the internally reflected beam for detecting the characteristics of the resonance that are dependent upon the reaction between the sample and the sensitive layer.

2. A sensor as claimed in claim **1** wherein the radiation from said source enters the block through a second, curved, surface.

3. A sensor as claimed in claim **2** wherein the center of curvature of said second, curved, surface lies on said first surface.

4. A sensor as claimed in claim **2** wherein said second surface is spherical.

5. A sensor as claimed in claim **2** wherein the input beam of electromagnetic radiation enters the block in a direction orthogonal to a tangent to the second, curved, surface at the point of entry.

6. A sensor as claimed in claim **2** wherein said detector means is positioned externally of said block, and wherein the internally reflected beam emerges from said block through a third surface of the block, said third surface being curved.

7. A sensor as claimed in claim **6** wherein the center of curvature of said third surface lies on said first surface.

8. A sensor as claimed in claim **2** wherein said transparent block takes the form of a hemisphere whose flat surface is said first surface and whose spherical surface includes said second surface, and wherein said part of said first surface is positioned at the center of the first surface.

9. A sensor as claimed in claim **2** wherein said transparent block takes the form of a semicylinder whose flat surface is said first surface and whose curved surface includes said second surface and wherein said part of said first surface is positioned on the longitudinal central axis of said first surface.

10. A sensor as claimed in claim 2 wherein said transparent block takes the form of a truncated hemisphere one of whose flat surfaces is said first surface and whose spherical surface includes said second surface, and wherein said part of said first surface is positioned at the center of the first surface.

11. A sensor as claimed in claim 2 wherein said transparent block takes the form of a truncated semicylinder one of whose flat surfaces is said first surface and whose curved surface includes said second surface, and wherein part of said first surface is positioned on the longitudinal central axis of said first surface.

12. A sensor as claimed in claim 1 wherein the detector means takes the form of a large-area detector positioned to collect the whole emergent beam.

13. A sensor as claimed in claim 1 wherein the detector means takes the form of an array of angularly spaced detectors positioned to collect the whole emergent beam.

14. A sensor as claimed in claim 1 wherein said sensitive layer takes the form of an antibody layer to be reacted with a sample containing a corresponding antigen.

* * * * *

US005229833A

United States Patent [19]

Stewart

[11] Patent Number: 5,229,833

[45] Date of Patent: Jul. 20, 1993

[54] **OPTICAL SENSOR**

[75] Inventor: **William J. Stewart**, Blakesley, England

[73] Assignee: **GEC-Marconi Limited**, Stanmore, England

[21] Appl. No.: **765,891**

[22] Filed: **Sep. 26, 1991**

[30] **Foreign Application Priority Data**

Sep. 26, 1990 [GB] United Kingdom 9020965

[51] **Int. Cl.**[5] **G01N 21/17**

[52] **U.S. Cl.** **356/364**; 356/128; 356/352

[58] **Field of Search** 356/364, 369, 370, 128, 356/352

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Primary Examiner—Richard A. Rosenberger

Attorney, Agent, or Firm—Jacobson, Price, Holman & Stern

[57] **ABSTRACT**

An optical sensor includes a resonant mirror device **1**, and a prism **2** disposed adjacent the device for coupling a beam of light into the device **1**. The device **1** and the prism **2** are mounted on a rotatable platform. A beam of light is produced by He-Ne laser **3** and is linearly polarized with equal TE and TM components by a polarizer **4** arranged at 45° to TE and TM axis. A lens **6** is arranged in the path of the linearly polarized beam of light for focussing the beam of light onto the device thereby providing simultaneously a range of angles of incidence at which the beam of light can be coupled into said device. The platform on which the device **1** and the prism **2** are mounted, is rotated to a position, at which the angle of incidence of beam of light coupled into the device is such that a resonance is excited in said device for at least one of said components. The beam of light reflected from the device is passed through an analyser **11** arranged at 90° to the polarizer **4**. On resonance of one or both components, one component is phase shifted to the other between 0 and 2 radians. The beam in this case will be elliptically polarized light and at least a component of light will be passed by the analyser **11** and will be projected as a bright band on a viewing plane. When the phase shift is radian, the beam is linearly polarized, but in the plane of the transmission axis of the analyser and so all the light is transmitted by the analyser.

8 Claims, 3 Drawing Sheets

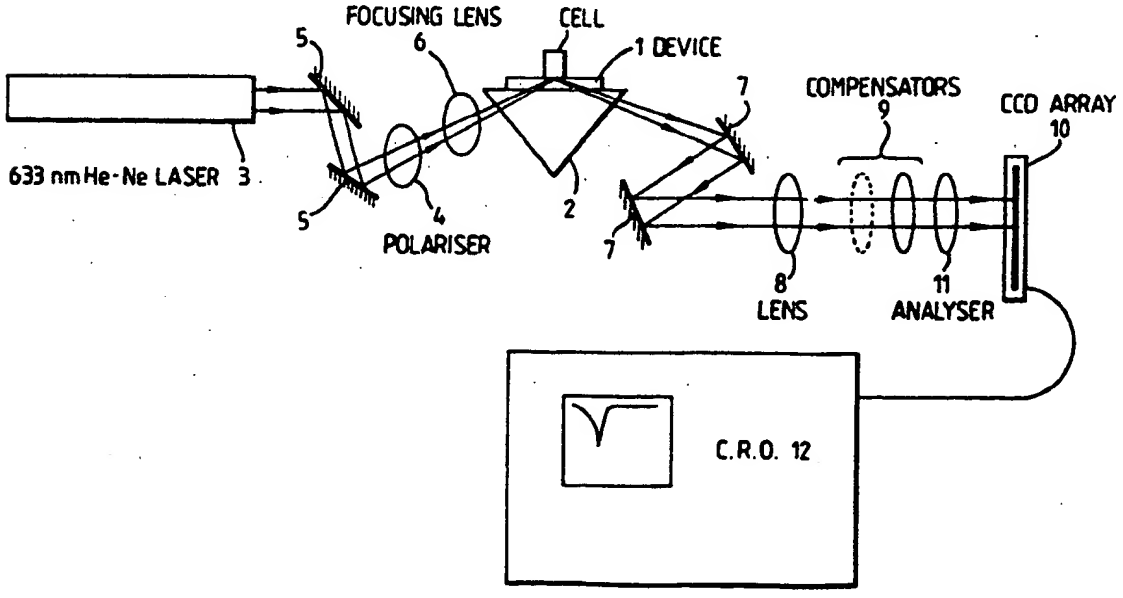
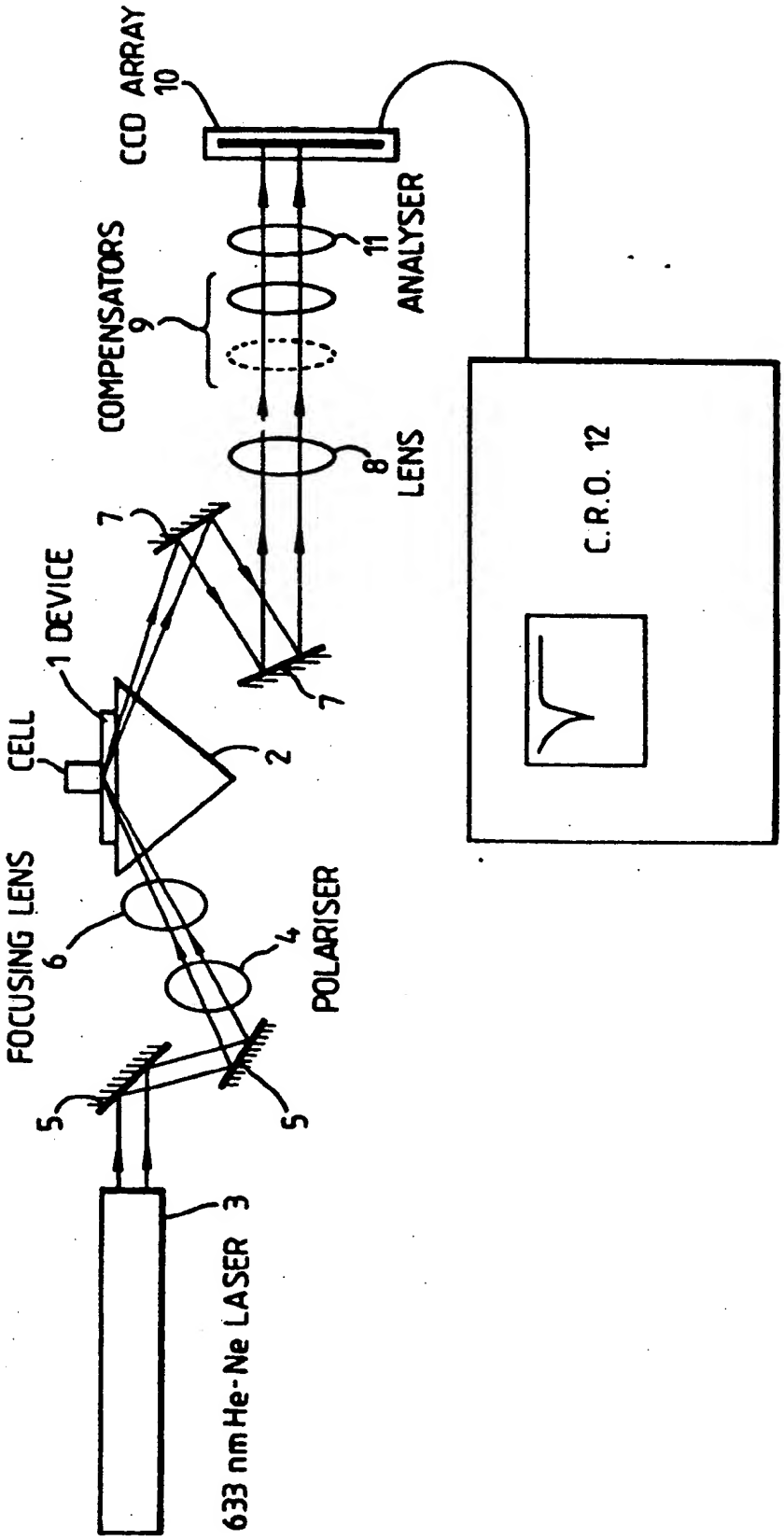


Fig.1.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10
11
ANALYSER
COMPENSATORS
9
8
LENS
7
7
1 DEVICE
CELL
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FOCUSING LENS
6
4
POLARISER
5
5
633 nm He-Ne LASER 3

Fig. 2.

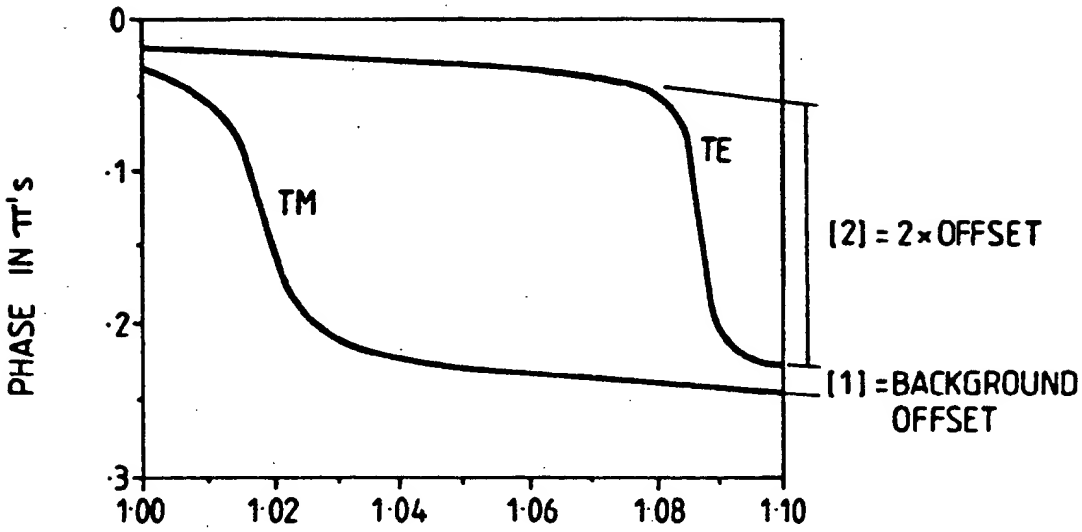
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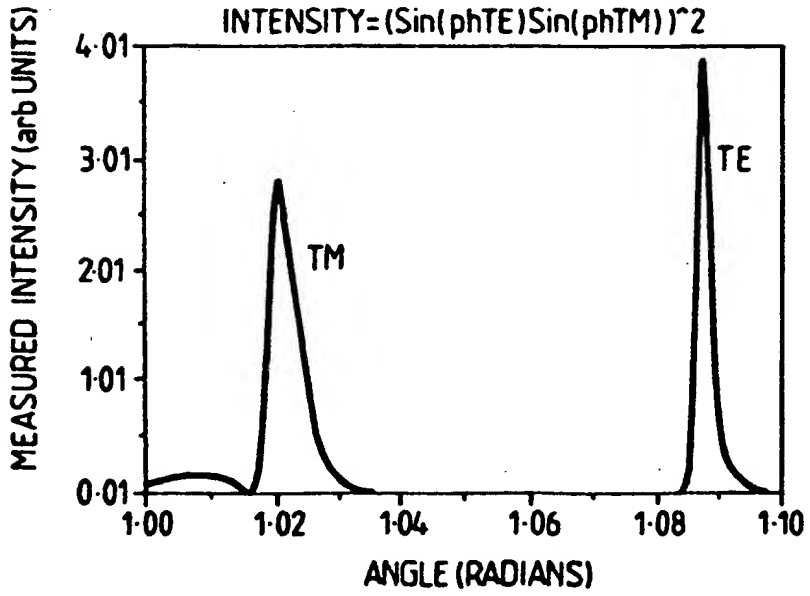
Fig. 3.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MEASURED INTENSITY (arb UNITS)
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1·06
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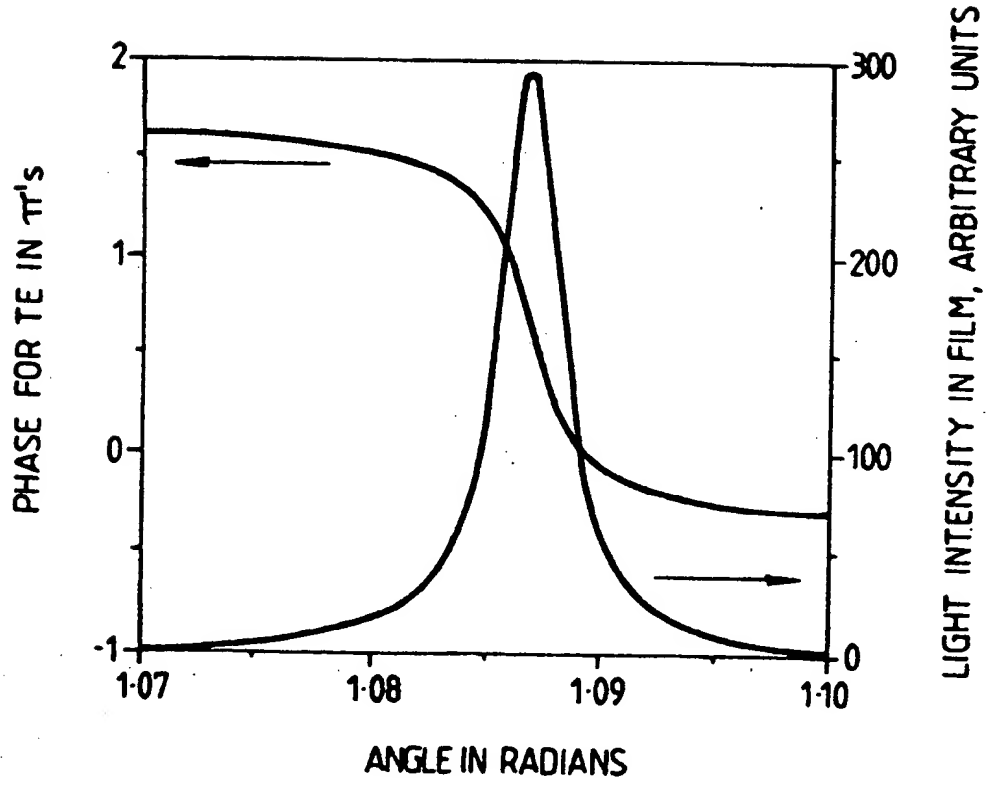
ANGLE (RADIANS)

Fig. 4.


PHASE FOR TE IN π's
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ANGLE IN RADIANS
300
200
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0
LIGHT INTENSITY IN FILM, ARBITRARY UNITS

OPTICAL SENSOR

The invention relates to an optical sensor and a method of testing a chemical sample.

There is need for immunological test devices for body fluids which satisfy the following criteria:

no sample preparation required,

small volume of fluid required (pinprick samples),

simultaneous testing of a number of analytes (3),

cheap, disposable testing elements,

short readout time,

desk top instrumentation.

Analysis systems satisfying these criteria will be of particular use in doctors surgeries, allowing them to carry out on the spot analyses. This has obvious advantages for the rapid diagnosis and treatment of disease, as well as being convenient for the patient. A number of technologies are currently under investigation for this application, including acoustic, amperometric, electrochemical and optical devices, in combination with biologial sensing layers consisting of monoclonal antibodies.

Immunosensing utilizes the method used by the body's immune system to identify foreign material. The body generates protein molecules, known as antibodies, which are capable of binding to the foreign material, the antigen, in a highly specific manner so aiding its identification and destruction. Most chemicals with molecular weights above 250 can induce the production of antibodies, with larger structures, such as bacteria and parasites, giving rise to multiple antibodies, each with a different binding site on the structure. Antibodies can therefore be used as a very powerful tool for the identification and detection of specific antigens.

A major use of these antibodies is in the clinical assay of body fluids for the detection of disease and abnormality, and in the monitoring of therapeutic drug treatment. The difficulty is to detect the antibody-antigen binding reaction. The antibody molecules are typically only 50-100 A in size and so are very difficult to detect by direct means. Most assays are carried out by attaching a label to the antibody molecule. Typical labels are radioactive or fluorescent molecules, or enzymes. Techniques using these allow for the accurate assays to be carried out, typically in the concentration ranges 10-6-10-15 molar. However they require reasonably large volumes of sample and involve complex procedures which need to be carried out by skilled personnel in a laboratory environment. To simplify these tests would represent a breakthrough in the use of clinical assay.

Optical techniques have been utilized for some time in the field of biosensors, monitoring reactions by measuring changes in absorption, fluorescence, scatter and refractive index, often remotely by the use of optical fibres. In particular, for immunosensing, great interest has been shown in evanescent optical sensors. These utilize the ability to immobilize antibodies in monolayers on a variety of substrates. The antibodies are immobilized onto the surface of a device so that the evanescent field of the light penetrates the antibody layer. Suitable devices include prisms, waveguides, gratings and fibres. Any binding reactions occuring at the antibody layer affects the evanescent field and hence the optical properties of the device. Using the evanescent wave as the sensing element has a number of advantges:

a) the light path is separated from the "wet" biochemistry,

b) the light probes only a surface layer, not seeing the bulk of the sample,

c) the volume of liquid required is only that which the evanescent field occupies—a few nanoliters.

In this way, the bulk of the sample does not interfere with the light either on its path to and from the sensing region or at the antibody sensing layer, so removing the requirement to separate out, for example, the cells in a blood sample. The small volume means that pinprick samples may be used, so reducing the discomfort caused to the patient, particularly where repeated measurements are to be made.

The binding reaction has been monitored using evanescent techniques which detect the absorption or fluorescence of the antibody molecules, either natural or via a label. More recently binding reactions have been sensed by measuring their effect on the phase of light passing through the bound layers, a consequence of changes in their thickneses and refractive indices. These include surface plasmon resonance sensors and waveguide devices, as well as the resonant mirror device.

Both the surface plasmon resonance (SPR) sensor and the resonant mirror sensor utilize resonance effects in thin films, plasmon resonance of the electrons in a metal (usually silver) film in SPR and optical resonance in a dielectric film in the resonant mirror. Light is coupled into the device and excites resonance at a particular incident angle. In the SPR device, as the resonating electrons radiate energy, light is absorbed by the metal film at resonance and the light reflected from the device is heavily attenuated. By monitoring the intensity of the reflected light as the incident angle is scanned, the position of the resonance is measured.

In the resonant mirror device, there is little attenuation of the light on resonance. However, the phase of the reflected light undergoes a shift of π radians, which may be measured using various interferometric techniques, so allowing a measure of resonance position.

In both devices the occurrence of a binding reaction within the evanescent region of the resonant field introduces an additional phase change. As a result, the angle at which resonance occurs is changed and this can be measured as a means of detecting the binding reaction.

These measurements may be made at a wide range of wavelengths (depending on device construction) and be used with any antibody-antigen reaction, without the use of labels. The devices are simple in construction and use and so lend themselves to use as cheap disposable sensors. However, it is believed that the resonant mirror device has a significant advantage over the SPR device. As the resonant structure in the resonant mirror is very low loss the resonance width is greatly reduced (in current devices the width is as low as 3 minutes of arc compared with typical SPR values of 1-1.5 degrees). This allows for much finer resolution of the angular shifts in resonance occuring on binding. When combined with the difference in the angle shift observed, we are left with an achieved increase in sensitivity of 10× that of SPR devices and optimization should enable increase into the region of 100×. In addition, as disclosed in the present invention, the resonant mirror possesses two distinct resonances, one for light polarized in the incident plane and one for light perpendicular to the plane. This allows the refractive index and thickness of the bound layer to be measured. SPR may only be excited by light polarized in the incident plane,

reducing the amount of information available and preventing these measurements being made.

Resonant mirror devices used in the present invention may be made from a variety of dielectric materials, using a variety of techniques. The devices can be fabricated by vacuum deposition of standard optical coating materials onto polished glass substrates, and consist of a low refractive index layer (e.g. magnesium fluoride or silica) covered by a thin high index layer (e.g. zirconia or titania). The polished glass substrates are then index matched to glass prisms to allow the coupling of light into the substrate. The devices are 10 mm×12 mm, so that the device may be illuminated with a wedge shaped beam allowing simultaneous monitoring of a number of areas across the device, each of which may be coated with a different antibody. This will allow multiple tests to be carried out on the sample, as required. For the disposable device it is preferred that the coupling element be part of the substrate. Substrates may be glass or polymer, using either prismatic shapes or grating structures to enable coupling. The dielectrc layers may be inorganic or polymer, deposited by vacuum, sol-gel or solvent deposition technique, depending upon the nature of the substrate.

When light is totally internally reflected from a boundary, it undergoes a phase change. The size of the phase change depends upon the refractive indices of the bounding materials, the wavelength of the light and the angle of incidence. Any changes at the boundary, such as antibody-antigen binding reactions, will alter the phase change. However for a simple high/low index boundary, the phase change is very slight and so the device is not very sensitive. In order to increase sensitivity, the resonant mirror device incorporates a resonant structure at the boundary, consisting of a high/low index pair of dielectric layers.

The layer pair acts rather like a Fabry-Perot cavity. One "mirror" of the cavity consists of the low index layer bounded by the two high index materials. Some of the light which is reflected from the lower boundary "tunnels", via the evanescent field, into the high index layer, a process known as frustrated total internal reflection. This layer therefore acts as a partially transmitting mirror, the degree of transmission being determined by the low index (or coupling) layer thickness. The second "mirror" of the cavity is the upper high index/low index boundary where total internal reflection occurs. This boundary is therefore 100% reflecting.

As with a Fabry-Perot cavity, resonance only occurs when the round trip phase delay between the mirrors is equal to a multiple of 2π radians. At resonance, the intensity of light in the cavity is high, at other times it is virtually zero. As the cavity has one totally reflecting boundary, all light is reflected from the resonant mirror device, both on and off resonance. However, the phase of the reflected light undergoes an additional change of π radians on resonance. It is the phase of the reflected light which is monitored in the resonance mirror device.

The incident angle at which resonance occurs is such that the total round trip phase delay, which consists of the distance travelled between the two boundaries of the high index layer together with the phase change on reflection at each boundary, is equivalent to a whole number of wavelengths. Any binding reaction occuring at the top surface changes the phase change on reflection at the upper boundary. To achieve resonance the incident angle must be changed to compensate for this. In a low loss system, the range of angles over which the resonant phase change occurs is very narrow and so very small changes in the resonant angle corresponding to small surface changes, can be detected.

The object of this invention is to enable the phase change on resonance occuring in an optical evanescent wave sensor device having a dielectric cavity to be observed more easily.

According to the invention there is provided an optical sensor comprising means for producing a beam of light with coherent TE and TM components, an optical evanescent wave sensor device having a dielectric cavity and arrange in the path of said beam of light for coupling said beam of light thereto, and an angular arranged to receive said components of the beam of light reflected from the device for producing a bright band and/or dark band, when one of said components excites resonance in said device.

Further according to the invention there is provided a method of testing a biochemicl sample, comprising providing an optical evanescent wave sensor device having a dielectric cavity and a sensing layer which is in at least in part sensitized by said sample, coupling a beam of light with coherent TE and TM components to said device to excite resonance in said device, and projecting said components of beam of light reflected from said device onto an analyser for producing a bright band and/or dark band.

By TE component is meant a component whose electric vector is perpendicular to the plane of incidence of the beam of light and by TM component is meant a component whose electric vector is in the plane of incidence of the beam of light.

The resonant mirror device which may be used in the optical sensor embodying the invention is simple in construction, consisting of a prism structure onto which one low and one high index dielectic film is deposited. These form a resonant cavity on the totally internally reflecting face of the prism. Antibodies for the species to be detected are immobilized onto this surface. Light is reflected off this surface within the prism and the phase of the reflected light is monitored. As the detected species binds to the antibody layer the angle at which resonance occurs changes, and this can be detected as a measure of the concentration of the detected species in the test sample. When the device is illuminated by a collimated polychromatic beam at an appropriate angle, only one wavelength will be on resonance. The wavelength at which resonance is excited may be monitored for testing the biochemical sample. This would require in the output optics means for wavelength demultiplexing, such as a diffraction grating or high dispersions prism. When the device is illuminated with a collimated beam from the tunable source, a resonance will occur at one particular wavelength. This wavelength can be monitored for testing the sample.

The device may be arranged in the path of the beam of light such that the resonance is excited for both of said components.

Preferably the input optic for the beam of light includes a lens arranged in the path of said beam of polarized light for focusing the beam of light onto the device, thereby providing simultaneously a range of angles of incidence at which said beam of light is coupled into said device.

Preferably the beam of light is linearly polarized with TE and TM components by a polarizer arranged in the path of the beam of light.

The polarizer may be arranged at 45° to the TE and TM transmission axes for providing equal components of TE and TM light and the analyser may be arranged at 90° to the polarizer for providing said bright band on to the viewing plane.

The sensor device may be a grating structure such as disclosed in PCT/GB89/01461, which includes a dielectric resonance cavity and an optical grating provided at one of the principal plane faces of the dielectric cavity for coupling light into the cavity.

Alternatively the device may be a resonant mirror device arranged in combination with coupling means for coupling light into said device. One example of such device and coupling means is disclosed in GB 2174802B.

Preferably said coupling means is a prism or diffraction grating mounted, together with said device, on a rotatable platform. The prism or diffraction grating together with said device may be mounted on a manually operable Vernier rotor stage so that angles of resonance can be measured. Preferably said resonant mirror device is fabricated on a surface of the prism or diffraction grating.

The output optics for the reflected light may include a compensator disposed adjacent said analyser to remove any phase difference which is introduced between the TE and TM components on total internal reflection and by birefringence in the device.

The bright band or dark band may be formed on a viewing plane at a position thereon corresponding to the angle of incidence at which resonance is excited in said device. The sensor may further include a CCD array connected to CRO for displaying said bright band or dark band on the screen thereof.

The beam of linearly polarized light with TE and TM components may be produced by a laser and a half wave plate disposed in the path of a beam of light produced by said laser.

The invention will now be described further by way of example with reference to the accompanying drawing in which:

FIG. 1 illustrates an optical sensor according to the invention for use with a resonant mirror device;

FIG. 2 is a graph of a phase shift on reflection versus angle for TE and TM reflected waves, note that the steps (2) associated with the resonances are both of 2πheight, and that there is, or may be, a small offset (1) which is corrected by the compensator;

FIG. 3 is a graph of intensity signals for TE and TM components v angle derived for the above FIG. 2 and;

FIG. 4 is a double plot showing the phase v angle for TE component as in FIG. 2, and the corresponding light intensity in the sensed film. This shows that the width of the phase step and the width of the resonance are the same. Arrows indicate the relevant ordinate for each graph.

Referring to the drawings, the resonant mirror device and the coupling device disposed adjacent thereto are mounted on a rotatable platform. Preferably the platform is a manually operable Vernier rotor stage so that angles of resonance excited in the device can be measured. The coupling device is a prism 2 as shown in the drawing. The prism 2 couples light into the device at an angle of incidence depending on the angular position of the rotatable platform relative to the beam of light. A diffraction grating may be used instead of a prism for coupling light into the device.

The input optics, provides a wedge beam of light allowing a range of input angles of incidence to be monitored. The input beam of light is produced by a laser 3 such as He-Ne laser with a wave length of 633 nm. The beam of light from the laser 3 is passed on to a polarizer 4 through light reflectors 5. The polarizer is arranged to produce a linearly polarized light with two components transverse electric (TE) and transverse magnetic (TM). The polarizer is set at 45° to the TE and TM transmission axes and thus provides equal components of TE and TM light. Alternatively the polarized beam of light with TE and TM components may be produced by a polarized laser and a half wave plate. TE component undergoes a phase change on reflection which is different compared with TM component.

As with all SP and resonant mirror devices there is a resonance at some angle '0', at which a plane wave incident on the structure will produce a maximum intensity in the resonant film. This maximum will typically be many (10^2+) times the intensity produced at other angles of incidence. All the light is reflected for any angle of incidence, so the resonance is detected because of the effect on the phase of the reflected wave. See for example FIG. 2, showing the resonance and the resulting phase. Note that the width '$\Delta\phi$' is the same for both curves. Because the materials affect the electric component of the light wave differently from the magnetic component, the resonance occurs at different angles for the TE and TM input waves. Assuming the angular separation between TE and TM resonances is large compared with their angular width, as is normally the case, the phase of each component could be shown as in FIG. 2. The step height for either TE or TM as shown in FIG. 2 is 2π. There may also be a 'background' phase difference between the curves at all angles 0 as shown in diagram 1 which is removed by the compensator. If this is done, remembering that a phase difference of $2\pi=0$ (see FIG. 4), then a curve like FIG. 3 is obtained. This could also be described as

$$\frac{1-\text{Cos}(\text{Phase } TE - \text{Phase } TM)}{2}$$

The linearly polarized light produced by a polarizer 4 is focused by a lens 6 on the device 1. The beam of light focused on the device is in the form of a wedge beam as shown in the drawing thus allowing a range of angles to be scanned simultaneously. The platform on which the prism 2 is mounted can be rotated so that the angles of incidence at which both components are coupled into the device can be adjusted. The prism is rotated so that the beam coupled into the device strikes the device at angles of incidence at which a resonance is excited for at least one of the TE and TM components. The prism may be rotated to a position where the resonance is excited for both of said TE and TM components.

In a resonant mirror device including a substrate of Corning glass with refractive index of 1.639, a coupling layer of magnesium fluoride of refractive index 1.38, a layer of zirconium oxide of refractive index 2.05 and an aqueous overlayer of refractive index 1.33, the TE resonance occurs at an angle of 60° 44' with a resonance width of 4.2' and TM resonance occurs at 56° 58' with a resonance width of 24'. On to this structure a layer of refractive index 1.436 and thickness 60 A corresponding to a monolayer of the protein immunoglobulin C (Tg) is provided. This produces a change in the resonant angle of 9.0' for TE and 4.5' for TM. The angles of resonance

may be measured by the pointer and scale provided on a Vernier rotor stage on which the prism 2 and the device 1 are mounted.

The reflected light from the device 1 is passed on to an analyser 11 through an output optics including reflectors 7, lens 8 and compensator 9. The analyser 11 is arranged at 90° to the polarizer. The two components are interfered at the analyser to allow the phase change on resonance to be detected. Off resonance both components undergo a similar phaseshift on total internal reflection and the relative phase between the components is adjusted by the compensator to give zero transmission through the analyser. This will apply for all angles except near resonance. Near resonance of either component, the phase shift between the TE and TM components will vary rapidly with angle, resulting in a maximum throughput of the analyser at resonance when all the light is transmitted. If a range of angle is scanned at once by using a wedge beam, a bright line on the dark background is projected onto the viewing plane. On rotating the analyser 90°, a dark band appears on a bright background on the viewing plane. A polarizing beamsplitter may be used to give both bright and dark band on the viewing plane. If the resonant angle changes, so does the position of the bright band and/or dark band on the viewing plane.

The lens 8 in the output optics is positioned at a focal length from the device and at a focal length from the viewing plane. This expands and collimates the beam whilst removing any diffraction effects. The compensator 9 consists of two quarter wave plates which are manually adjusted to remove any phase difference which is introduced between the TE and TM components on total internal reflection and by birefringence if the optical path.

The bright band of light from the analyser may be projected on a Charge Coupled Device array 10 (CCD array); the position of the bright band on the CCD array corresponds to the angle of resonance. Thus a shift of position of the bright band on the CCD array corresponds to a shift in the resonance angle. The output of CCD is passed on to a Cathode Ray Osciloscope 12 (CRO) for display on the screen. Calibrating the division across the CRO screen using the Vernier on which the prism is mounted allows the calculation of the angular width of the resonance.

I claim:

1. An optical sensor for testing a biochemical sample, the optical sensor comprising means for providing a beam of light, a polarizer arranged in the path of the beam of light to linearly polarize said beam of light with coherent TE and TM components, an optical evanescent wave sensor device arranged in the path of said polarized beam of light, said sensor device having a dielectric cavity and a sensing layer which is at least partly sensitized by said sample, the beam of light being polarized at approximately 45° at the surface of the cavity, coupling means for coupling said beam of light with coherent TE and TM components to said sensor device and an analyzer arranged to receive said components of the beam of light reflected from the device for producing at least one of a bright band and a dark band, when at least one of said components excites resonance in said device.

2. A sensor as claimed in claim 1, including a lens arranged in the path of said beam of light for focusing the beam of light onto the device, thereby providing simultaneously a range of angles of incidence at which said beam of light is coupled into said device.

3. A sensor as claimed in claim 1, in which said coupling means is one of a prism and a diffraction grating.

4. A sensor as claimed in claim 3, in which said device is fabricated on a surface of one of said prism and said diffraction grating.

5. A sensor as claimed in claim 4, in which one of said prism and said diffraction grating together with said device is mounted on a manually operable Vernier rotor stage so that angles of resonance can be measured.

6. A sensor as claimed in claim 1, including a compensator disposed adjacent said analyzer to remove any off resonance phase difference which is introduced between the TE and TM components.

7. A sensor as claimed in claim 1, including a CCD array for monitoring one of said bright band and said dark band.

8. A method of testing a biochemical sample, the method comprising providing a beam of light, linearly polarizing said beam of light with coherent TE and TM components, arranging in the path of said polarized beam of light, an optical evanescent wave sensor device having a dielectric cavity and a sensing layer which is at least partly sensitized by said sample, the beam of light being polarized at approximately 45° at the surface of the cavity, coupling said beam of light with coherent TE and TM components to said sensor device to excite resonance in said device for at least one of said components and arranging an analyzer to receive said components of the beam of light reflected from the device for producing at least one of a bright band and a dark band.

* * * * *

United States Patent [19]

Finlan et al.

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[45] **Date of Patent: Sep. 10, 1991**

[54] **BIOLOGICAL SENSORS**

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[73] Assignee: **Amersham International PLC**, Little Chalfont, England

[21] Appl. No.: **299,564**

[22] Filed: **Jan. 18, 1989**

[30] **Foreign Application Priority Data**

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[51] **Int. Cl.**[5] **G01N 21/00**; G01N 21/55

[52] **U.S. Cl.** **422/82.11**; 422/82.05; 422/57; 422/68.1; 422/58; 436/164; 356/318; 356/445

[58] **Field of Search** 422/82.05, 82.11; 356/318, 445

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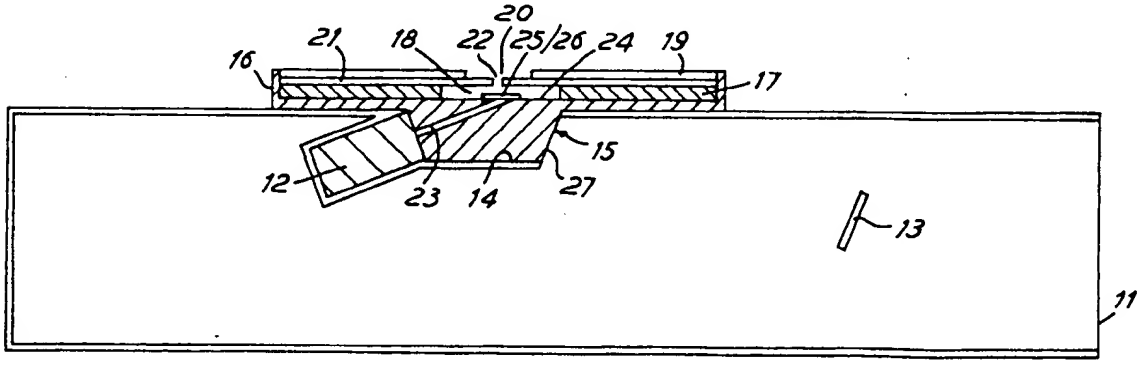
Primary Examiner—Robert J. Warden
Assistant Examiner—Theresa A. Trembley
Attorney, Agent, or Firm—Wenderoth, Lind & Ponack

[57] **ABSTRACT**

A biological sensor which utilizes the phenomenon of surface plasmon resonance to detect the refractive index change which occurs when two components—for example antibody and corresponding antigen—react with one another. Surface plasmon resonance takes place at the sloping exit surface of an optical waveguide such as a fiber optic **23**. The input end of fiber optic **12** is connected to a light source **12**. A layer **25** of metal is applied to the sloping exit surface so as to cause total internal reflection of the light proceeding down the fiber optic. Reflected light is detected by a detector **13**. A sensitive, for example antibody, layer **26** is applied to the metal layer. Sample (not shown) reacts with layer **26** in such a way that the refractive index changes. Provided conditions are correct, this variation in refractive index can be monitored in detector **13** by virtue of the surface plasmon resonance which occurs in the area of total internal reflection.

15 Claims, 5 Drawing Sheets



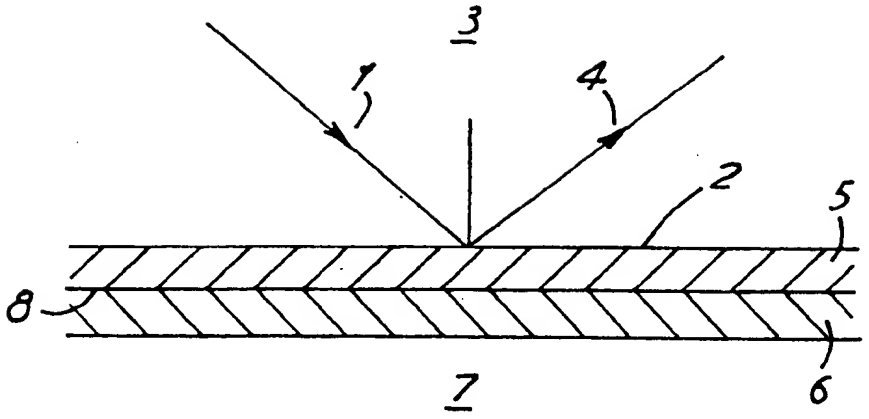


FIG. 1

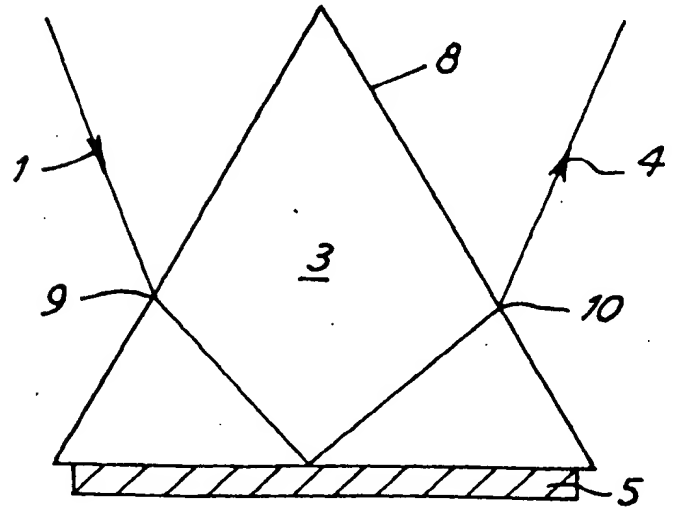


FIG. 2

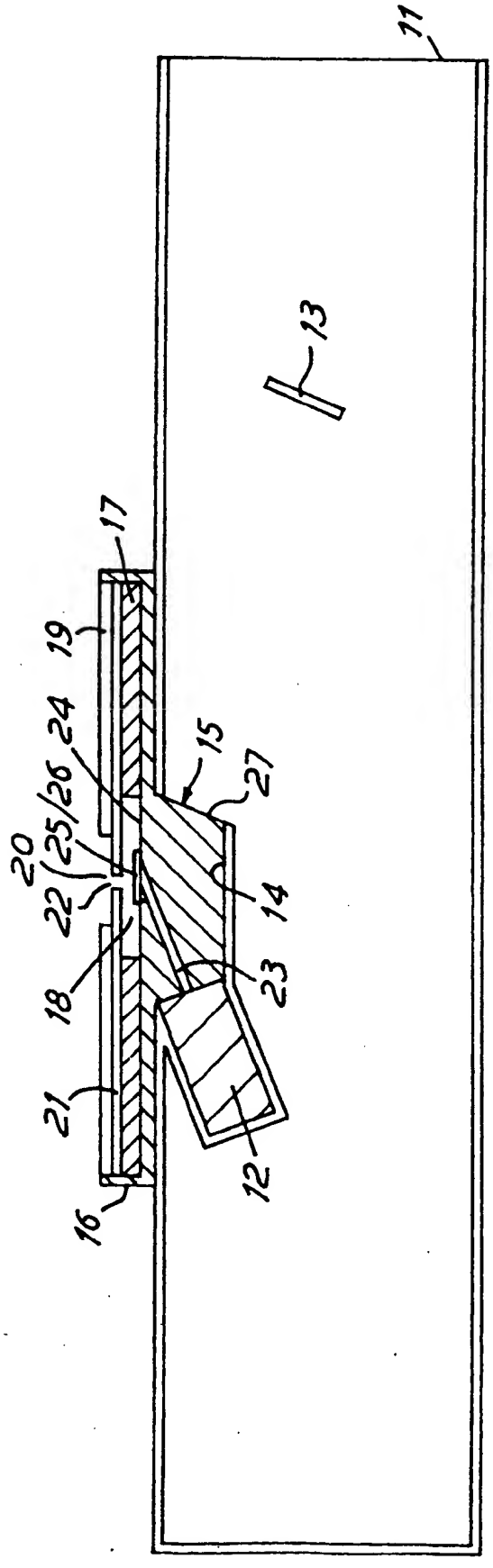


FIG. 3

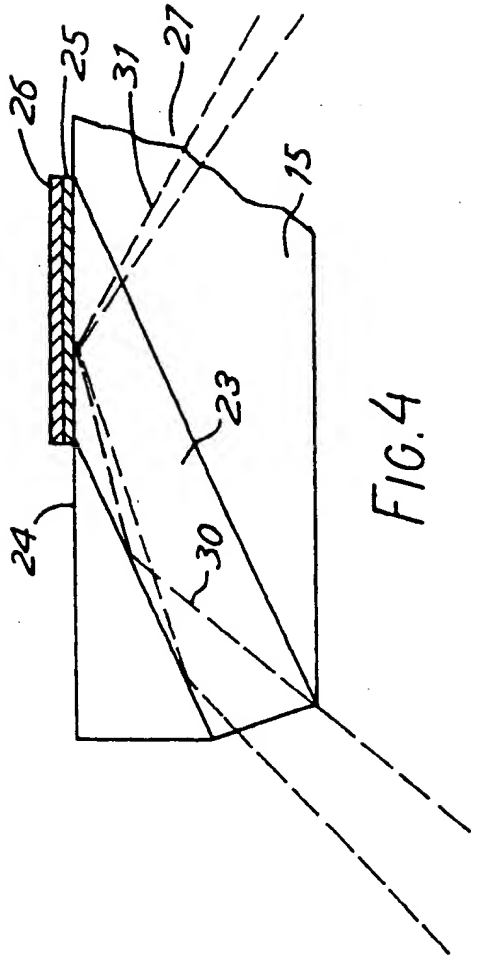


FIG. 4

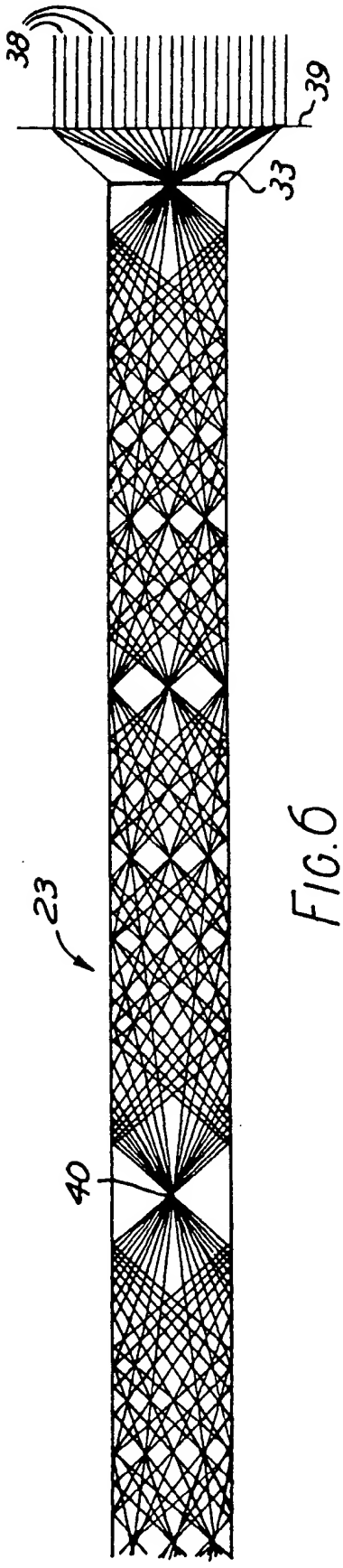


FIG. 6

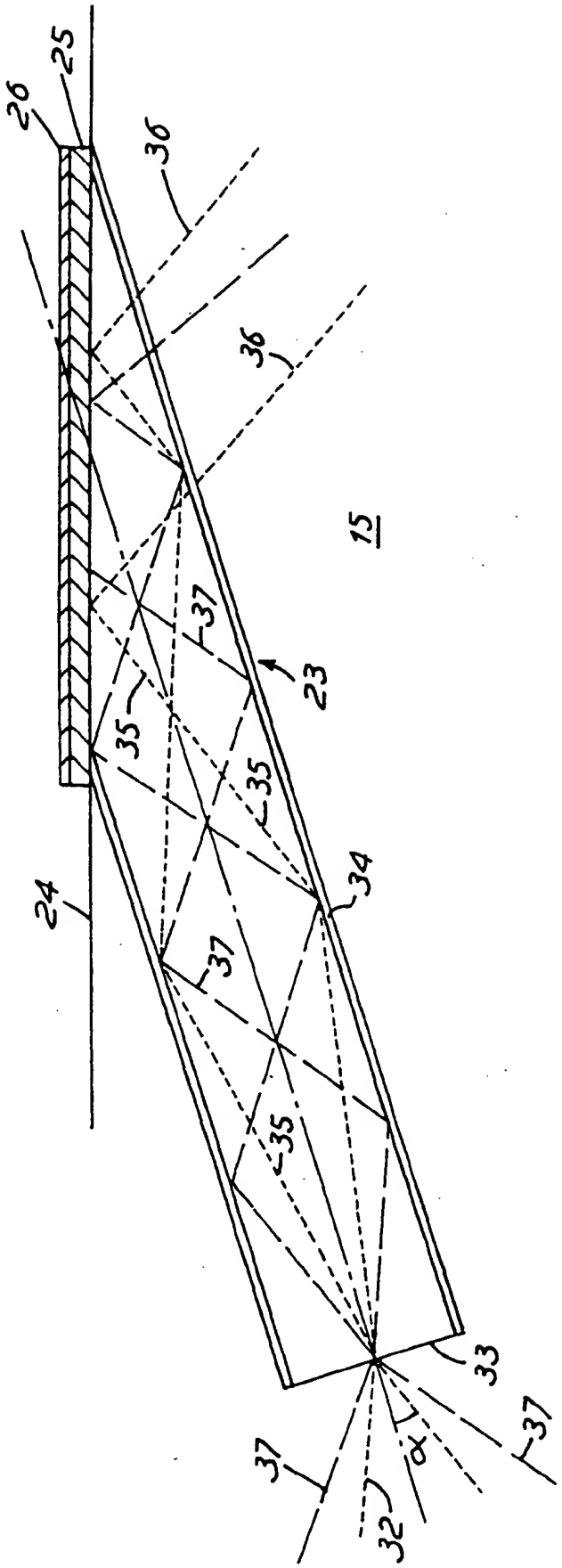


FIG.5

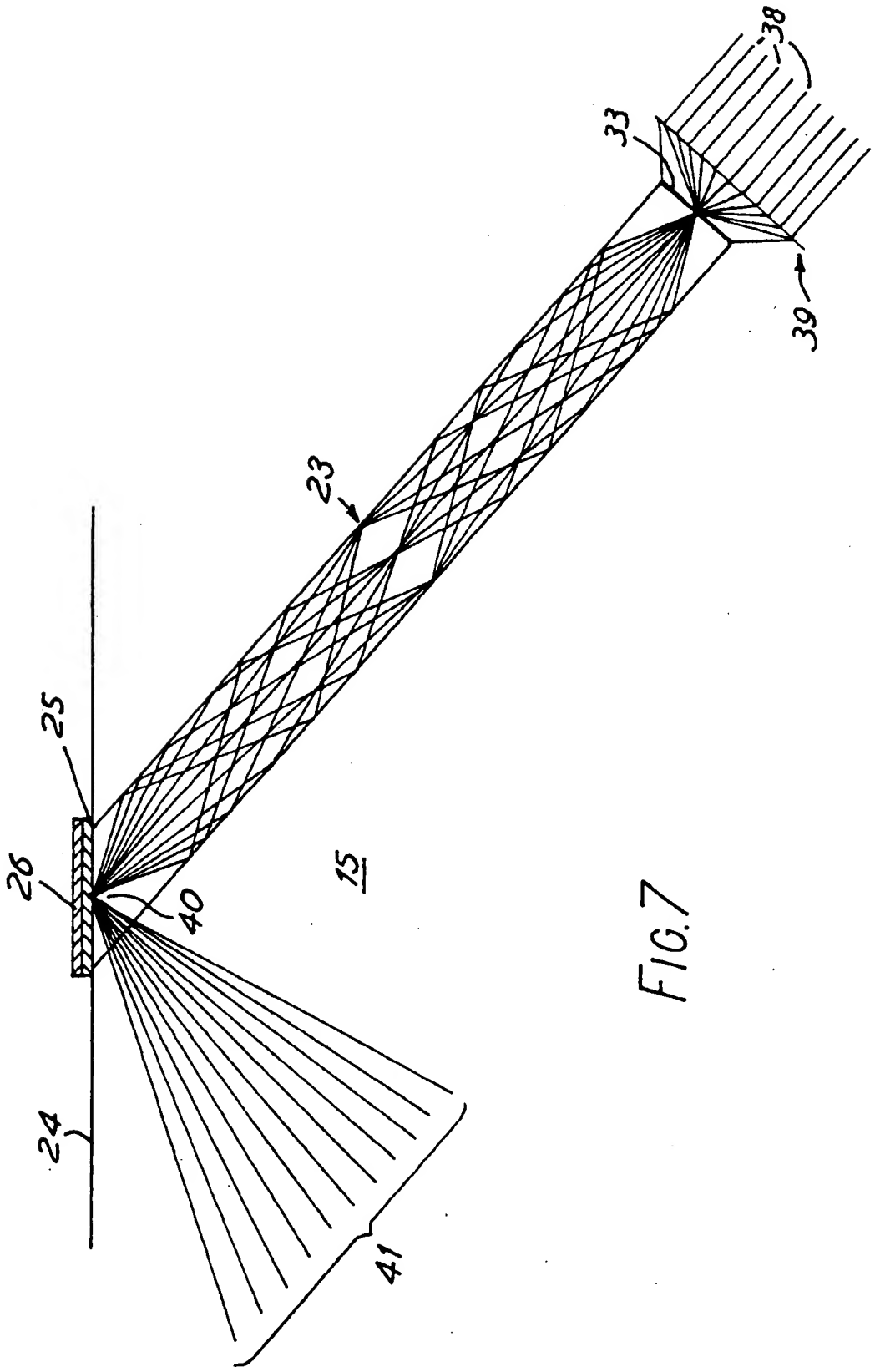


FIG. 7

BIOLOGICAL SENSORS

This invention relates to sensors for use in biological, biochemical and chemical testing and in particular to immunosensors used to monitor the interaction of antibodies with their corresponding antigens.

When antibodies are immobilized on a surface, the properties of the surface change when a solution containing a corresponding antigen is brought into contact with the surface to thus allow the antigen to bind with the antibody.. In particular, the change in the optical properties of the surface can be monitored with suitable apparatus.

The phenomenon of surface plasmon resonance (SPR) can be used to detect minute changes in the refractive index of the surface as the reaction between the antigen and the antibody proceeds. Surface plasmon resonance is the oscillation of the plasma of free electrons which exists at a metal boundary. These oscillations are affected by the refractive index of the material adjacent the metal surface and it is this that forms the basis of the sensor mechanism. Surface plasmon resonance may be achieved by using the evanescent wave which is generated when a light beam is totally internally reflected at the boundary of a medium, e.g. glass, which has a high dielectric constant. A paper describing the technique has been published under the title "Surface plasmon resonance for gas detection and biosensing" by Lieberg, Nylander and Lundstrom in Sensors and Actuators, Vol. 4, page 299. Illustrated in FIG. 1 of the accompanying drawings is a diagram of the equipment described in this paper. A beam 1 of light is applied from a laser source (not shown) onto a surface 2 of a glass body 3. A detector (not shown) monitors the internally reflected beam 4. Applied to the external surface 2 of glass body 3 is a thin film 5 of metal, for example gold or silver, and applied to the film 5 is a further thin film 6 of organic material containing antibodies. A sample 7 containing antigen is brought into contact with the antibody film 6 to thus cause a reaction between the antigen and the antibody. If binding occurs the refractive index of the layer 6 will change owing to the increased size of the antibody molecules and this change can be detected and measured using the surface plasmon resonance technique, as will now be explained.

Surface plasmon resonance can be experimentally observed, in the arrangement of FIG. 1, by varying the angle of the incident beam 1 and monitoring the intensity of the internally reflected beam 4. At a certain angle of incidence the parallel components of the light momentum will match with the dispersion for surface plasmons at the opposite surface 8 of the metal film. Provided that the thickness of metal film 5 is chosen correctly there will be an electromagnetic coupling between the glass/metal interface at surface 2 and the metal/antibody interface at surface 8 which results in surface plasmon resonance and thus an attenuation in the reflected beam 4 at that particular angle of incidence. Thus, as the angle of incidence of beam 1 is varied, surface plasmon resonance is observed as a sharp dip in the intensity of the internally reflected beam 4 at a particular angle of incidence. The angle of incidence at which resonance occurs is affected by the refractive index of the material against the metal film 5—i.e. the antibody layer 6—and the angle of incidence corresponding to resonance is thus a direct measure of the state of the reaction between the antibody and the antigen. Increased sensitivity can be obtained by choosing an angle of incidence half way down the reflectance dip curve, where the response is substantially linear, at the beginning of the antibody/antigen reaction, and then maintaining that angle of incidence fixed and observing changes in the intensity of the reflected beam 4 with time.

Known systems of the type described with reference to FIG. 1 utilize a prism as the glass body 3. A diagram showing this arrangement is given in FIG. 2 which is simply an experimental set up intended to demonstrate surface plasmon resonance. The prism is shown under reference 8 and has applied to its undersurface a thin film 5 of metal. Light 1 from a laser source (not shown) is incident on the prism where it is refracted at point 9 before entering the prism. The internally reflected beam 4 is likewise refracted (at point 10) upon exiting from the prism.

One problem with the prism is that, as the angle of incidence is changed, either by moving the source, or rotating the prism, or both, the point on surface 2 at which the incoming beam is incident moves. Because of inevitable variations in the metal film 5 and the coating 6 of antibody, the angle of incidence which results in resonance changes as this movement occurs, which in turn introduces a further variable factor into the measurement and thus makes comparisons between the initial, unbound, state and the bound state of the antibody layer 6 less accurate. In addition to this, the system shown in FIG. 2 is not realistic for mass production, where cheap and readily disposable components are required.

According to the present invention there is provided a sensor for use in biological, biochemical or chemical testing, said sensor comprising an optical waveguide having an input end and an output end, a source of electromagnetic radiation whose output is applied to the input end of said optical waveguide, and wherein said output end of the optical waveguide is cut off at an angle to its axis to provide a sloping end face, means for monitoring the radiation from said source which is internally reflected at said face, a layer of metallic material applied to said sloping face, a layer of sensitive material applied to the metallic layer, and means for introducing onto the sensitive layer so as to react therewith a sample to be analysed, the arrangement being such that the radiation incident at said face of the optical waveguide causes surface plasmon resonance to occur, the characteristics of which resonance, as detected by said monitoring means, is dependent upon the reaction between the sample and the sensitive layer. Normally the radiation is in the visible or near-visible region, and this will be assumed throughout the present specification.

The term "optical waveguide" as used herein is intended to cover any transmission line for electromagnetic radiation within or near the optical range, and in which the wave propagates along the waveguide by means of repeated internal reflections off the wall of waveguide. Examples of such waveguides include the well-known fiber optic, on which the remainder of the present specification concentrates, but may also include rectangular section waveguides such as microscope slides along which, from edge to edge, light may be transmitted by means of repeated interval reflections off the major surfaces of the slide.

Fiber optics rely for transmission of light on repeated internal reflections at the walls of the fiber, the light

taking a zig-zag course as it proceeds along the fiber. In order to ensure that such internal reflection takes place, fiber optics may be clad with a material having a lower refractive index than that of the material of the fiber. Commonly the fiber itself is made from glass, and the cladding of plastics material having a lower refractive index.

In order to mechanically support the fiber, it is preferred that the fiber be embedded in a block of transparent material. It is necessary that the material be transparent in order to allow it to pass light internally reflected at the sloping end of the fiber optic and which thus emerges from the fiber optic to be intercepted by the monitoring means. If the refractive index of the material of the block is chosen suitably, it can act in place of the cladding in ensuring internal reflections along the walls of the fiber optic. This can be useful where the integrity of the cladding is suspect, or where the cladding is not present at all.

The optics can be arranged in various different ways. For example, the radiation source may incorporate means for focussing the radiation (i.e. light) onto the input end face of the fiber optic. In these circumstances, the characteristics of the fiber optic are such that the sloping output face becomes illuminated with a range of angles of the input light. Thus the input beam effectively becomes several beams incident upon the glass/-metal interface over a range of angles. The equipment can be chosen so that the range of angles spans the angle of dip corresponding to surface plasmon resonance. The corresponding internally reflected beam is likewise effectively several beams and may be monitored by a large area detector, or by an array of angularly spaced detectors positioned to collect the whole emergent beam. Thus the detectors can encode the information from the whole of the dip within milliseconds.

An equivalent effect can be obtained by arranging that the focussing means focusses the radiation onto the output surface of the fiber optic—in other words, onto the glass/metal interface. Here again the input beam effectively spans a range of input angles which can be simultaneously monitored as described above.

Although the layer applied to the metal film is assumed herein to be an antibody layer for use in immunoassays, it will be seen that any sensitive layer whose refractive index changes upon an event occurring can be used to thus provide a sensitive detector having a wide variety of applications in the fields of biology, biochemistry and chemistry. For example, the antibody could be replaced with other analyte specific binding entities such as DNA probes.

The metal film material is commonly silver or gold, usually applied by evaporation. The film needs to be as uniform as possible in order to cater for minute movement in the point of incidence of the incoming beam. It is assumed that a structured metal film will give the best resonance and there are various ways in which the glass body can be pretreated to improve the performance of the metal film and in particular to control the natural tendency of such films to form discontinuous islands:

1. Immersion in molten metal nitrates and other molten salts. This has the effect of introducing ions into the surface in a manner which can be structured and which can act as foci for island formation.
2. Ion bombardment of cold or hot glass to introduce nucleating sites. The removal of the more mobile ions has been demonstrated to reduce the thickness at which the evaporated film becomes continuous.
3. Electroless plating or electroplating over lightly evaporated films (0 to 100 angstroms thick). Electroless plated films survive to a greater thickness than evaporated films and could form more stable nuclei for subsequent coating.
4. Evaporating on to electroless plated films. The electroless plated films have a stronger tendency to an island structure and to bigger islands with greater spacing than evaporating films. This could be of advantage in tuning to light of a prescribed wavelength.

Coating performance can also be improved by:

1. Controlling the glass surface temperature during coating. Using a higher temperature substrate increases the islands' size and the spacing between them and conversely.
2. Evaporating in the presence of a magnetic or electrostatic field or electron emission device to control the ion content of the vapor stream. The state of charge of the substrate is known to affect the island structure.
3. Controlling the angle of incidence of the evaporated vapor stream relative to the glass surface. The mobility of the evaporated atoms and hence their ability to form bigger islands is greater when the momentum of the atoms relative to the glass surface is increased.

In order that the invention may be better understood, an embodiment thereof will now be described by way of example only and with reference to the accompanying drawings in which:

FIGS. **1** and **2** are diagrams of known experimental arrangements for demonstrating the surface plasmon resonance effect;

FIG. **3** is a diagrammatic side view of an embodiment of a sensor according to the present invention;

FIG. **4** is a diagrammatic side view of part of the sensor of FIG. **3**, on a larger scale, showing an example of the path of the light rays;

FIG. **5** is a diagrammatic side view of part of the sensor of FIG. **3**, on a still larger scale, showing an alternative example of the path of the light rays;

FIG. **6** is a diagrammatic side view showing the propagation of annular beams of light along a fiber optic; and

FIG. **7** is a view similar to FIG. **5**, but illustrating the use of annular beams, such as in FIG. **6**.

Referring to FIG. **3**, the apparatus comprises a casing **11** carrying a laser diode and lens assembly **12** and a large area imaging detector **13** such as a diode array, charge couple device (ccd) or similar. The source produces a collimated input beam of electromagnetic radiation. The frequency of the radiation must be such as to result in the generation of surface plasmon waves and in practice will be within or near the visible region. Suitable sources include an infra red diode laser, but an ordinary light source, such as an LED (light emitting diode), with suitable filters and collimators, could be used.

The diode and lens assembly **12** is situated to one side of a well **14** formed in the top surface of the casing **11**. This well is adapted to receive and locate a disposable test assembly built around a block **15** of radiation transparent material. The upper portion of the block is formed in the shape of a shallow tray having sides **16**, and which contains three circular discs arranged one on top of another. The lowermost disc **17** is made of absorbent material and has a central through-aperture defining an active zone **18**. The upper disc **19** has a central

aperture intended to define a well 20 into which a sample to be tested is placed. The middle disc 21 has a central aperture 22 which is of a size to cause liquid in well 20 to travel through by capillary action into the active zone 18.

The lower portion of the block 15 is shaped to locate securely into the wall 14. The lower portion has cast or moulded therein a fiber optic 23 which extends from the output of the laser diode and lens assembly 12 to the surface 24. The output end of the fiber optic is cut off at an angle to define a sloping exit face which is substantially coplanar with the surface 24 of the block 15. This sloping exit face can be seen more clearly in FIGS. 4 and 5. The exit face is ground and polished for maximum accuracy.

The fiber optic is made of transparent material such as glass or plastics material which has a refractive index less than that of the surrounding block 15. Alternatively in the case of a clad fiber optic, the refractive index of the material of the block should be the same as or less than that of the cladding. If the cladding is suspect, a lower refractive index is best, as this ensures the internal reflections necessary for the light to travel along the fiber optic.

Applied to the sloping exit face of the fiber optic is a metal film layer 25, for example of silver, on top of which is applied a further layer 26 of a sensitive material whose refractive index changes as the test progresses. The sensitive layer 26 may for example be an antibody layer. The thickness of the metal layer 25 is such as to maximize the surface plasmon resonance reflectance dip when coated with the sensitive layer 26 and immersed in a typical test light from well 20—e.g. serum.

In order to reduce the effects of discontinuities in the layers 25 and 26, both of which can cause inaccuracies, it is desirable that the layers 25 and 26 are kept small in area, restricted in fact to the area of the sloping exit face of the fiber optic. The diameter of the fiber optic is typically 100 microns, but can span a large range of diameters depending on the application. Diameters less than 10 microns are not normally used because of increased difficulty in coupling light into the fiber optic. However, these smaller diameters could be used if special coupling techniques are employed, such as wedge or grating couplers.

As will be explained in more detail, during operation of the equipment, light from the laser diode and lens assembly is coupled into the fiber optic and is internally reflected at the sloping exit face to emerge from the fiber optic into the material of block 15, through which latter it travels before finally exiting through a window 27 in the well 14 to ultimately impinge on the sensitive area of the detector 13.

In order to use the apparatus a sample to be tester, containing an antigen capable of binding with the antibody molecules in layer 26 is placed in the well 20 and passes through apertures 22 by capillary action. Emerging from aperture 22, the liquid sample commences to flow radially outwards in all directions towards the absorbent disc 17, passing as it does so the antibody layer 26. The sample adjacent the layer 26 is thus being constantly replenished during the course of the test, which ensures maximum sensitivity.

As the sample flows past the layer 26 any antigen within the sample capable of binding with the antibody in layer 26 will do so, thus altering the refractive index of layer 26 as the reaction proceeds. This change in refractive index is continuously monitored during the test by directing along the fiber optic 23 the light beam from assembly 12. Provided that conditions are correct—in particular the angle of incidence at the point of incidence on the fiber optic exit face is correct—the application of the light will result in the generation of a plasmon wave, thus extracting energy from the input beam and causing an attenuation in the intensity of the output beam at a particular angle of incidence. The input beam is arranged such that the mid-angle of the range of angles of the input beam is approximately halfway down the reflectance dip, as described above, and the test is carried out at a constant angle of incidence, monitoring the intensity of the reflected beam above and below this mid point level. This gives a linear and highly sensitive output.

The initial reflectance dip which is chosen for setting up the angle of incidence should be the dip which results when some neutral or buffer solution is passed through the cell, or when the sample under test is passed through the cell but before any reaction thereof has taken place. In connection with the latter method, which is currently preferred, it is to be noted that, as sample begins to flow past the active zone adjacent layer 26 the refractive index does not start to change immediately due to the antibody/antigen reaction. There is thus sufficient time to take an initial reading with the unreacted sample flowing past, which reading can be utilized, using feedback circuitry, to rapidly adjust the angle of incidence to an appropriate value half way down the reflectance dip so that the rest of the test can be performed at this fixed angle.

With particular reference to FIGS. 4 to 7, we now consider three ways of optically operating the equipment. In the first way, shown in FIG. 4, the diode and lens assembly 12 is such as to provide an incident light beam 30 which is brought to a focus at the surface 24—i.e. on the sloping exit face of the fiber optic. The incident light beam thus covers a range of input angles which can be arranged to cover the angles of incidence which are known to produce the dip in the internally reflected beam, or to cover that part of the dip—for example just one side thereof—which is to be used for measurement.

The internally reflected beam, shown under reference 31, is divergent and escapes from the fiber optic due to its large angle of incidence with the wall of the fiber optic. After leaving the fiber optic the reflected beam travels through the cladding, if any, and then into the material of block 15. Due to the different refractive indices, there is bound to be some refraction of the beam, but this should be fairly minimal. Any such refraction can to a certain extent be compensated for as the reflected beam emerges from the block 15 into the air space within the casing 11. To this end the window 27 can be shaped such as shown in FIG. 4.

The reflected beam leaving window 27 is intercepted by the detector 13 which gives an output signal for analysis by external circuitry (not shown).

In the alternative arrangement, shown in FIG. 5, the diode and lens assembly 13 is such as to provide a light beam 32 focussed onto the input face 33 of the fibre optic 23. FIG. 5 also shows, by way of illustration, the use of a clad fiber optic, the layer 34 of cladding being of a material having a lower refractive index than that of the fiber optic itself.

The dotted lines, reference 35, show the multiple reflection progress of the beam down the fiber optic until it reaches the sloping exit surface. Here, internal

reflection takes place and an output beam **36** results. This latter beam passes through the cladding, then through the block **15** into the interior of the casing **11** where it is intercepted by the detector **13**, as before.

The angle of the input beam is chosen to suit the circumstances; the dotted lines **37** represent the largest limit of the input angle beyond which internal reflection at the walls of the fiber optic will not take place, and transmission along the fiber optic is not possible.

In a further alternative arrangement, shown in FIG. **7**, the input light beam takes the form of a series of separate, spaced coaxial beams **38** of annular section. Such a composite beam can be produced, for example, by sputtering rings of obscuration, coaxial with a solid input light beam (not shown), onto a transparent plate (not shown) onto which the solid beam is incident at right angles.

Before discussing the FIG. **7** arrangement in detail, reference will be made of FIG. **6** which shows a diagram of the ray paths of a composite beam such as described above along a fibre optic **23**. The composite beam is first brought to a focus at the input face **33** of the fiber optic by means of a suitable lens **39**. For the fiber optic shown, lens **39** will be a circular lens, for planar optics (such as the above-mentioned microscope slide), a cylindrical lens will be used. The front face of the lens **39** is coated with emulsion in a pattern of concentric rings coaxial with the lens axis. This produces, from a solid input beam, a series of concentric coaxial ring-section beams—note that, for the sake of explanation, the input beam entering lens **39** is shown as already divided into separated ring-section beams. The number of rings of emulsion, and hence the number of separate annular beams generated, will be dictated by the required angular definition at the detector **13**. In a typical arrangement, with a lens diameter of 2 cm and a fiber optic diameter of 1 mm, the number of rings would typically be **256**.

As the light propagates into the material of the fiber optic it internally reflects off the fiber optic walls in the manner described above, and takes up a distinct pattern, as illustrated. In particular, it will be noted that, at a point **40** along the fiber optic, the separate beams making up the composite input beam come to a focus. Beyond the focal point **40**, the ray pattern repeats itself and will thus result in further focal points (not shown) along the fiber optic length. The distance d of the first focal point **40** from the input face **33** is given by:

$$d = \frac{2 \times \text{diameter of fiber}}{\text{increment of gradient}}$$

Increment of gradient refers to the tangent of the angle of the incoming beam relative to the axis, converted into a gradient. For example, for a typical 10 micron diameter fiber at 0.001 gradient, the distance d is 20 mm. It will be seen that the exact distance d is dependent upon the number of separate annular input beams—the greater the number, the greater the distance.

In the arrangement of FIG. **7**, the sloping exit face of the fiber optic is formed at such a position that it passes through the focal point **40** (or any of the later focal points, if a long fiber optic is required). The resultant divergent output beam **41** which passes into the block **15** is directed, as previously, to the detector **13**.

The advantage of this arrangement is that, because at the point of internal reflection on the sloping exit face the incident beams are brought to a focus, the size of the active zone is kept to a minimum, thus reducing errors caused by irregularities in the layers **25** and **26**.

In a further embodiment of the invention (not shown), the block **15** incorporates more than one fibre optic **23**, each of these latter being illuminated with a common light source, or separate light sources. Each of these extra fiber optics terminate in a sloping exit face coplanar with surface **24** and spaced from adjacent fiber optics, and are covered with a metal layer **25** and sensitive layer **26**, as before. It will be seen that, by this means, several distinct active areas can be defined, at each of which an analysis of a common sample can be carried out. As well as carrying out multiple tests simultaneously on one sample, this arrangement enables reference tests to be set up. Alternatively, by providing separate sample feeding arrangements—i.e. separate wells **20** etc.,—the same test can be carried out simultaneously on a number of different samples.

In a still further embodiment of the invention (not shown) the fiber optic is replaced by a plate of transparent material such as plastics or glass—a microscope slide will be suitable. One edge of the sheet is formed with said sloping exit face, and the light is inputted into the opposite edge. An advantage of using a sheet such as this is that multiple input beams can be applied to the input edge of the sheet and, if correctly adjusted, will propagate separately and independently along the sheet to the opposite edge. In conjunction with several distinct active areas, in the manner described above, this can enable simultaneous analysis of a number of different samples, or can enable a number of different tests (using, for example, different antibodies) to be carried out simultaneously on a common sample.

The use of the fiber optic to couple the light to the surface at which plasmon resonance takes place enables the area of the active zone to be minimized which reduces errors due to discontinuities in the metal and antibody films. In addition to this the physical size of the expensive antibody film is kept to a minimum. The system lends itself to mass production, and the fiber itself and its associated components should be cheap enough to be disposable, in the manner described above.

Although the fiber optic **23** is shown as being straight, there is no reason why a curved fiber optic could not be used if the physical constraints of the apparatus require it. For example, in a multiple-fiber apparatus where each fibre optic is illuminated with a separate light source, it may be found more convenient to use curved fiber optics from the (relatively large) light sources to the (relatively closely spaced) active zones.

The refractive indices of the block **15** and fibre **23** must be chosen with some regard to the quality of the surface plasmon resonance which results: in particular, we are looking for a steep slope on at least one side of resonance, or preferably on both sides since then the slopes can be algebraically added to give a higher amplitude output signal, and thus an improved signal to noise ratio. Generally speaking the refractive index of the fiber is chosen in relation to that of the sensitive layer **26** immersed in typical sample fluid to give a good resonance; the refractive index of block **15** is thence chosen in relation to that of the fiber to give the required optical properties.

We claim:

1. A sensor for use in biological, biochemical or chemical testing, said sensor comprising:

an optical waveguide having an input end and an output end and along which radiation may propa-

gate by means of internal reflections off its internal surfaces,

a source of electromagnetic radiation whose output is applied to the input end of said optical waveguide, and

wherein said output end of the optical waveguide is cut off at an angle to its axis to provide a sloping end face,

wherein said radiation is input to the waveguide as a focussed beam so that the beam is incident at said end face as a spread of angles, and

wherein the angle of the sloping end face is such as to (1) cause the incident beam to be totally internally reflected at said face, and (2) cause the thus-reflected beam to be incident on the wall of the waveguide at an angle sufficiently great for it to exit from the waveguide without being subject to further internal reflection,

means for monitoring the radiation from said source which is internally reflected at said face,

a layer of metallic material applied to said sloping face,

a layer of sensitive material applied to the metallic layer, and

means for introducing onto the sensitive layer so as to react therewith a sample to be analyzed,

the arrangement being such that the aforesaid spread of radiation incident at said face of the optical waveguide is such as to include that angle at which surface plasmon resonance occurs, and allows the changing characteristics of the resonance to be monitored, which characteristics, as detected by said monitoring means, are dependent upon the reaction between the sample and the sensitive layer.

2. The sensor as claimed in claim **1** wherein said optical waveguide is formed of solid material transparent to the radiation in use, and along which the radiation propagates by means of internal reflections off its internal surfaces.

3. The sensor as claimed in claim **2** wherein the optical waveguide is a fiber optic.

4. The sensor as claimed in claim **2** wherein the optical waveguide is a rectangular slab of transparent material, such as a microscope slide.

5. The sensor as claimed in any one of claim **2** to **4** wherein the material of the waveguide is clad with a material having a refractive index which is lower than that of the waveguide material.

6. The sensor as claimed in any one of claim **2** to **5** wherein the material of the waveguide, and its cladding (if any) is embedded in a block of transparent material.

7. The sensor as claimed in claim **6** wherein the material of said block has a refractive index which is lower than that of the waveguide material.

8. The sensor as claimed in either one of claims **6** or **7** wherein said block is arranged to receive light internally reflected off said sloping end face and passing out of said waveguide medium, and wherein said block has an output face through which such internally reflected light passes, to be incident on said monitoring means.

9. The sensor as claimed in claim **8** wherein said output face is curved, having a center of curvature coincident with the point at which the radiation is incident on said end face.

10. The sensor as claimed in any one of claims **2** to **9** or a further including focussing means for focussing the radiation from the source onto the sloping end face.

11. The sensor as claimed in any one of claims **2** to **9** or **1** further including focussing means for focussing the radiation from said source onto the input of the optical waveguide.

12. The sensor as claimed in claim **11** wherein the optical waveguide is of circular cross-section, further including means for forming the radiation from the source into a series of separate radially spaced, coaxial beams of annular section.

13. The sensor as claimed in claim **11** wherein the optical waveguide is of rectangular cross section, further including means for forming the radiation from the source into a series of separate spaced planar beams.

14. The sensor as claimed in either one of claim **12** or **13** wherein the axial length of the optical waveguide is such that all of the separate input beams come to a common focus at the end face.

15. The sensor as claimed in any one of claims **2** to **14** or **1** including one or more further optical waveguides illuminated by a common source of radiation or by respective separate sources of radiation to enable testing of multiple analytes in a single sample, or multiple samples, to be carried out simultaneously.

* * * * *

United States Patent [19]

Stewart

[11] **Patent Number: 4,857,273**

[45] **Date of Patent: Aug. 15, 1989**

[54] **BIOSENSORS**

[75] Inventor: **William J. Stewart, Blakesley, United Kingdom**

[73] Assignee: **Plessey Overseas Limited, Ilford Essex, United Kingdom**

[21] Appl. No.: **848,680**

[22] Filed: **Apr. 4, 1986**

[30] **Foreign Application Priority Data**

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[51] **Int. Cl.**[4] **G01N 21/77**

[52] **U.S. Cl.** **422/68**; 350/96.12; 350/96.15; 350/96.19; 350/96.34; 422/55; 422/57; 422/69; 435/287; 435/291; 435/808; 436/805

[58] **Field of Search** 436/805; 422/57, 58, 422/69, 68, 55; 350/96.12, 96.15, 96.17, 96.19, 96.34; 435/287, 291, 808

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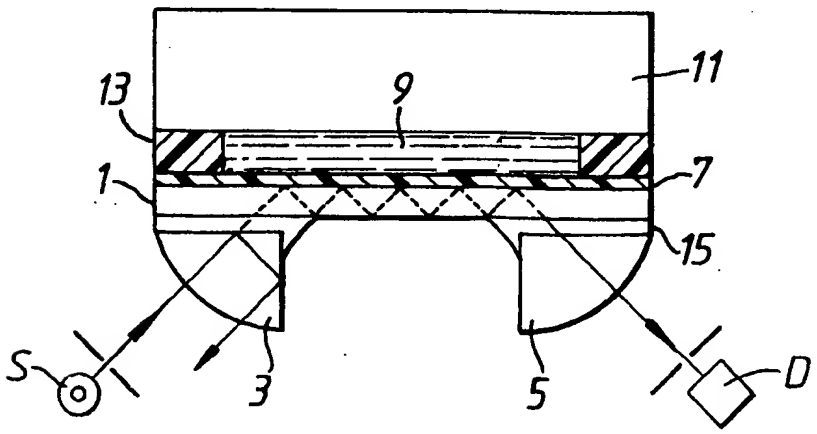
Primary Examiner—Robert J. Hill, Jr.

Attorney, Agent, or Firm—Fleit, Jacobson, Cohn, Price, Holman & Stern

[57] **ABSTRACT**

A biosensor includes an optically dense body provided with a coating sensitized to a specific assay species, and an input and output coupling structure. Light signal response is enhanced by incorporating a partially reflecting, partially transmitting medium between the coupling structure and the optically dense body having a lower refractive index. The thickness of such medium is chosen so that light may be coupled by frustrated total internal reflection and to enable the medium to serve as a resonant mirror.

6 Claims, 1 Drawing Sheet



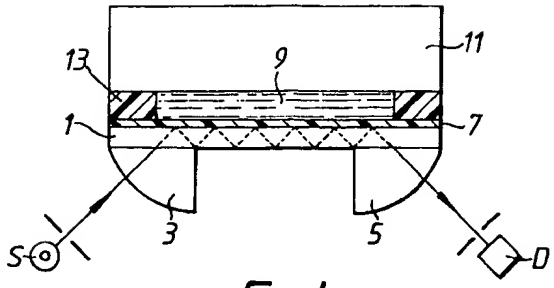


FIG. 1.
PRIOR ART

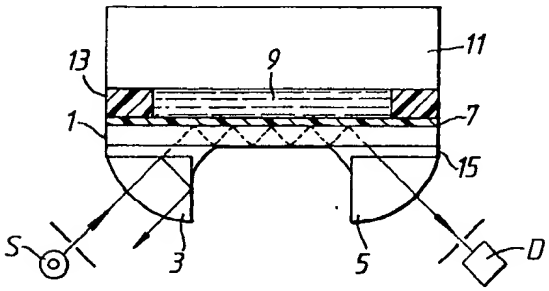


FIG. 2.

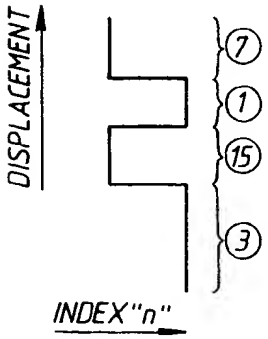


FIG. 3a.

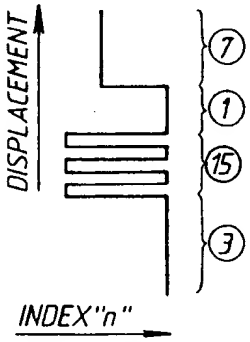


FIG. 3b.

BIOSENSORS

BACKGROUND OF THE INVENTION

1. Field Of The Invention

The present invention concerns biosensers, i.e. sensors for detecting and/or monitoring or quantifying the presence and/or behavior of specific species in test fluid samples. The invention is applicable to the following: immunoassays, i.e.; the detection of antibodies, antigens, or hormones in blood samples; pollution monitoring; and, to the monitoring of clinical diagnostic reactions involving enzymes and the like.

2. Description of Related Art

A recent article entitled "Detection of Antibody—Antigen Reactions at a glass-liquid Interface as a Novel Optical Immunoassay Concept", (1984), R. M. Sutherland et al (Proceedings of 2nd Optical Fibre Conference (Stuttgart 1984) page 75) describes a biosensor in which an antibody species is covalently immobilized onto the surface of a planar or fibre-optic waveguide. The reaction of immobilized antibody with antigen in a sample solution is detected using the evanescent wave component of a light beam which has been totally internally reflected many times within the waveguide. The evanescent wave has a characteristic penetration depth of a fraction of a wavelength into the aqueous phase, thus optically interacting with substances bound to or very close to the interface and only minimally with the bulk solution.

Reference is also made to our United Kingdom Patent Application BG 2156970A published Oct. 16, 1985, which discloses optic-waveguide biosensors and a similar technique. The content of that disclosure is incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention is intended to enhance biosensor response for a given beam power.

In accordance with the invention there is provided a biosensor comprising:

an aplically dense body having a coating sensitized for a given assay species; and Light coupling means adjacent to the optically dense body, to direct light into and out of the same; wherein a light reflecting and partially transmissive medium is interposed between the aplically dense body and the light coupling means, so that the coating, the optically dense body, and the light reflecting and partially transmitting medium together provide a mirrored resonant cavity.

In the above defined construction the combination of coating, body, and reflecting medium have the propertis of a mirrored resonant cavity. The power of radiation within this cavity is thus enhanced relative to the power of the input beam and the power of the interactive evanescent wave extending into the sensitive coating is likewise enhanced, thereby improving device sensitivity to species absorbed by the coating.

The reflecting partially transmissive medium may be realized by a thin single layer of relatively low refractive index transparent material. Alternatively, it may be realized by a dielectric multilayer structure.

BRIEF DESCRIPTION OF THE DRAWINGS

In the accompanying drawings:

FIG. 1 is an illustrative cross-section view of a known biosensor;

FIG. 2 is an illustrative cross-section view of a biosensor modified in accordance with this invention; and

FIG. 3 illustrates schematic refractive index profiles for a modified biosensor incorporating

(a) a single layer reflector; and

(b) a multi-layer structure reflector, respectively.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Embodiments of the invention will now be described, by way of example only, with reference to the drawings accompanying this specification.

In FIG. 1 there is shown a known biosensor in which light from a source S is directed into a planar waveguide 1 by means of a first coupling prism 3 and propagated by multiple total internal reflection to exit by means of a second coupling prism 5 where it is directed onto a light detector D. The external surface of the waveguide 1 is provided with a sensitized organic is coating 7. The latter coating exposed to a sample liquid 9 which is contained by means of a flow cell 11 and gasket 13 arrangement. In the coating 7, antibody material is covalently immobilized and this responds to any specific antigen material in the sample liquid to which it is exposed. The waveguide is of fused quartz material and this provides a large differential in optical density between the quartz waveguide 1 (high refractive index n_1) and the adjacent coating 7 (low refractive index n_2). Light is totally internally reflected within the body of the waveguide 1, with a portion of the optical power, propagating as an evanescent wave in the coating medium 7. The binding of antigen by the immobilized antibody is monitored by a resultant increase in the light absorption measured at the detector D.

In the inventive modification shown FIG. 2, a partially transmissive light reflecting layer 15 of relatively low refractive index material—for example an evaporated or sputtered layer of magnesium fluoride-is interposed between the coupling prisms 3, 5 and the planar waveguide 1. This construction may be used in conjunction with an infra-red light injection laser as light source S—typical light wavelength 0.8μ. Similar apparatus may be used for visible light and for ultra-violet light, but in the latter case a layer 15 of alumina or similar material would be used—typical light wavelength 270 nm. Light is coupled to the waveguide 1 by frustrated total internal reflection, and the thickness of the interposed layer 15 is chosen accordingly. The refractive index profile for this assembly of media—coating 7, waveguide 1, reflector 15 and coupling member 3—is shown FIG. 3(a). As can be seen, the waveguide 1 is isolated by media 7, 15 of lower refractive index. Incident light coupled to the waveguide is thus resonantly trapped between the reflecting layer 15 (highly reflecting but partially transmitting) and the coating/waveguide interface 7/1. The power level is high in this region. The light subsequently leaks back into the bulk medium, the second coupling member 5, after which it is monitored by the photodetector D. The evanescent wave in the coating 7 will interact with any adsorbed species in this layer 7 and in turn will modify the absorption and phase shift of the light beam monitored. The latter is enhanced by this resonant effect.

As an alternative modification, the single interposed layer 15 of the assembly shown in FIG. 2 may be replaced by a dielectric multilayer structure 15. Such dielectric multilayer structures are per se well known to persons of oridinary skill in the art and thoroughly

understood as to function and use so that the details thereof form no part of the present invention. A typical index profile for this modified assembly is shown in FIG. 3(*b*). This further modification has the advantage of allowing a relaxation in coupling constraints. As an example, a multilayer structure providing 90% reflection and 10% transmission provides a factor x10 enhancement of power within the resonant cavity.

The present of assay species may be detected and/or monitored by measuring changes in the absorbtion or polarazation of the monitored light beam. The interaction will depend on the frequency and angle of incidence of the light beam. Thus source S and detector D may be singular components mechanically scanned over a range of angles, or may each comprise an extended array, each component being electronically addressed to simulate a scan. Alternatively, the source S and detector D may be set up in an optimal static configuration.

Having described the invention and the manner in which it may be performed, I/We claim:

1. A biosensor comprising: an optically dense body having a coating sensitized for a given assay species, light coupling means positioned in operative adjacent relation to the optically dense body for directing light into and out of the same, and means operatively associated with the light coupling means for esablishing a mirrored resonant cavity within the optically dense body by frustrated total internal reflection, including a light reflecting and partially transmissive medium operatively interposed between the optically dense body and the light coupling means.

2. The biosensor as claimed in claim 1, wherein the light reflecting and partially transmissive medium comprises a single layer of material of a thickness establishing light coupling by frustrated total internal reflection, said material having a refractive index lower than that of the optically dense body.

3. The biosensor as claimed in claim 2, wherein the single of material layer is magnesium fluoride.

4. The biosensor as claimed in claim 2, wherein the single layer of material is alumina.

5. The biosensor as claimed in claim 1, wherein the light reflecting and partially transmissive medium comprises a dielectric multilayer structure.

6. A monitoring system comprising a light source, a photodetector, a biosensor comprising: an optically dense body having a coating sensitized for a given assay species, light coupling means operatively positioned adjacent to the optically dense body for directing light into and out of the same and means operatively associated with the light coupling means for esablishing a mirrored resonant cavity within the optically dense body by frustrated total internal reflection, including a light reflecting and partially transmissive medium operatively interposed between the optically dense body and the light coupling means, said light source and the photodetector being positioned relative to the biosensor in optically coupled resonance relation to each other.

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